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Advancing forest structure-function relationships: linking above- and belowground structure to soil respiration

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
at Virginia Commonwealth University

by

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Abstract

ADVANCING FOREST STRUCTURE-FUNCTION RELATIONSHIPS: LINKING ABOVE- AND BELOWGROUND STRUCTURE TO SOIL RESPIRATION

By Laura Jane Hickey, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

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Major Director: Christopher Gough, PhD, Biology

Forest canopy structural features are robust indicators of aboveground carbon (C) cycling processes, but whether aboveground vegetation structures are similarly coupled with belowground structure and function is poorly understood. Variation in the soil-to-atmosphere C flux, or soil respiration (R_s), is influenced by a suite of biotic and abiotic factors, including soil temperature, soil moisture, and root biomass. However, whether canopy structure is tied to soil respiration through its simultaneous influence over these drivers is not known. We assessed relationships between measures of above- and belowground vegetation density and complexity, and evaluated whether R_s is linked to remotely sensed canopy structure through pathways mediated by established biotic and abiotic mechanisms. Our results revealed that, at stand-scale, canopy rugosity–a measure of complexity–and vegetation area index were coupled to soil respiration through their effects on light interception, soil microclimate, and root biomass density, but this connection was much stronger for complexity. Measures of canopy and root complexity were not spatially coupled at the stand-scale, as canopy but not root complexity increased over successional timescales. Our findings demonstrate that remotely sensed canopy complexity can be used to infer spatial variation in soil CO2 efflux, and that this relationship is

grounded in established biotic and abiotic pathways. The broader inference of linking soil respiration to remotely sensed canopy complexity requires additional multi-site investigation, which is possible given burgeoning open data from ecological networks and satellite remote sensing.

Vita

Laura Jane Hickey was born on August 27, 1997 in Fairfax, Virginia. She graduated *summa cum laude* from Colonial Forge High School located in Stafford County, Virginia in 2015. She received dual Bachelor of Science degrees in Biology and Environmental Studies with a minor in Chemistry from Virginia Commonwealth University, Richmond, Virginia in 2019, where she was awarded Outstanding Undergraduate Student in Ecology from the Biology department. Her publications include “Contrasting Development of Canopy Structure and Primary Production in Planted and Naturally Regenerated Red Pine Forests”, “The forte data R package: open-science datasets from a manipulative experiment testing forest resilience”, and “Fire after clear-cut harvesting minimally affects the recovery of ecosystem carbon pools and fluxes in a Great Lakes forest”.

Introduction

Forest canopy structure is strongly tied to *aboveground* microclimate and production (Hardiman et al. 2011, Gough et al. 2019), but whether canopy structure drives *belowground* carbon (C) cycling processes through its effects on soil microclimate and root structure is not known. Spatial variation in the soil-to-atmosphere C flux, or soil respiration, is influenced by a suite of canopy-influenced biotic and abiotic factors. For example, canopy structure affects light transmission (Ishii et al. 2004, Atkins et al. 2018), soil microclimate (Flerchinger & Pierson 1991, McCarthy & Brown 2006, Tanaka & Hashimoto 2006, Forrester et al. 2012, Cai et al. 2021), and root biomass (Hopkins et al. 2013, Suchewaboripont et al. 2015), each of which constrain the spatio-temporal dynamics of soil respiration (Raich & Schlesinger 1992, Wang et al. 2017). In addition, canopy effects on root carbohydrate supply and exudation (Litton et al. 2007) may couple canopy structure to soil respiration by affecting the metabolic activity of roots and microbes (Sun et al. 2017). The interacting biotic and abiotic pathways coupling canopy structure to soil respiration, however, have not been fully elucidated, limiting integrative understanding of these above and belowground structure-function interactions.

The few studies relating canopy structure to soil respiration have emphasized linkages with aboveground vegetation area or canopy reflectance (Tanaka & Hashimoto 2006, Katayama et al. 2009, Bréchet et al. 2011, Forrester et al. 2012, Ma et al. 2014, Shi et al. 2016, D'Andrea et al. 2020, Cai et al. 2021), and have not considered canopy structural complexity, a potent predictor of aboveground C cycling processes. "Structural complexity" measures generally summarize the heterogeneity of aboveground vegetation distribution (Hardiman et al. 2011), and can be derived from terrestrial light detection and ranging (LiDAR) measurements of horizontal and vertical vegetation distribution (Lim et al. 2003, Kane et al. 2010, Atkins et al. 2018). Aboveground vegetation complexity metrics such as canopy rugosity (*sensu* Hardiman et al. 2011) are strongly correlated with stand-to-regional variation in forest primary production through their effects on light acquisition and light-use efficiency (Hickey et al. 2019, Gough et al. 2019). Similarly, conventional root structural metrics summarizing vertical biomass distributions and densities (Zhou & Shangguan 2007, Grienwald et al. 2021) are correlated with spatial variation in soil respiration (Pregitzer et al. 1998). Spatial mirroring of above- and belowground forest structure, such as coherence of gaps in root and canopy density, has been identified (Hardiman et al. 2017), but whether such relationships extend to canopy-root *complexity* measures is not known (Fig. 1). Therefore, while segments of the pathway connecting canopy structure and soil respiration provide a strong foundation of knowledge, a more integrative understanding of above-belowground interactions is required to enhance mechanistically-grounded inference of soil respiration using terrestrial remote sensing (Cavender-Bares et al. 2021).

We assessed relationships between measures of above- and belowground vegetation structure and determined whether soil respiration is linked to remotely sensed canopy structure through its influence on root spatial distribution and soil microclimatic factors with established mechanistic ties to soil CO_2 efflux. To accomplish this goal, we analyzed forest stands that span a range of aboveground structures, which have been shaped by successional development and disturbance, and are distributed across a glacial drift landscape in northern Michigan (Scheuermann et al. 2018, Wales et al. 2020). Specifically, our objectives (O) were to: evaluate successional changes in above- and belowground structural measures summarizing vegetation density and complexity (**O1**); assess how aboveground structural measures relate to soil microclimate and root structure

(**O2**); and determine whether soil microclimate and root structure mediate relationships between canopy structure and soil respiration (**O3**). Our corresponding hypotheses (H) were: above and belowground stand-scale structural properties will develop similarly over succession (Hardiman et al. 2017) (**H1**); canopy structural metrics summarizing heterogeneity in the distribution and density of vegetation in multiple dimensions (e.g., canopy rugosity) will better predict spatial variation in mean soil temperature, moisture, and root structure than those summarizing vegetation density alone (e.g. vegetation area index) (Scheuermann et al. 2018) (**H2**); and canopy structure will be linked to soil respiration through mediating relationships with biotic and abiotic variables that mechanistically tie canopy structure to C-cycling (i.e. light absorption) or drive soil respiration (i.e. soil temperature, soil moisture, and fine roots) (**H3**).

Methods

Study site

Our study took place at the University of Michigan Biological Station (UMBS) in northern Lower Michigan, USA (45.558, -84.677). This landscape is covered by secondary forests, regrown following clear-cut harvesting and subsequent fires in the late 19th and early 20th centuries. With the goal of encompassing a breadth of above- and belowground structural variation, we conducted our study in two separate 100-yr chronosequences and three late successional forest stands spanning productivities, compositions, canopy complexities, and leaf area indices representative of forests throughout the broader Great Lakes - Laurentian Mixed Forest ecological province (Nave et al. 2017, Scheuermann et al. 2018, Wales et al. 2020). The two chronosequences differed by disturbance history at the time of stand establishment. The first chronosequence includes four, 1-ha “cut and burn” stands that were clear-cut and burned in 1936, 1954, 1980, or 1998; and the second consists of four “cut only” clear-cut (but not burned) 1-ha stands in 1911, 1952, 1972, or 1987. Early successional stands were populated mostly by bigtooth aspen (*Populus grandidentata*) and paper birch (*Betula papyrifera*), with mid-successional (~100-yr-old) stands transitioning to red oak (*Quercus rubra*) and red maple (*Acer rubrum*) dominance. Late successional stands were >130-yrs-old and represented distinct plant functional types: deciduous broadleaf forests (DBF) dominated by *Q. rubra* and *A. rubrum*, evergreen needleleaf forest (ENF) dominated by *Pinus resinosa*, and a mix of both deciduous and needleleaf species (MIX; *Pinus strobus, P. resinosa, Q. rubra, P. grandidentata*). In each of the ll stands, sampling occurred in two or three, 0.1 hectare plots (n = 29 total plots). Plots were considered the experimental unit of analysis.

Canopy and root structure

We used a portable below-canopy LiDAR (PCL) system equipped with an upward-facing, near-infrared laser to derive two categories of aboveground structure with known ties to ecosystem functioning: vegetation area index (VAI) and canopy rugosity (Gough et al. 2019). Canopy data were collected at maximum leaf out in July 2021. VAI describes the number of leaf and woody vegetation layers present per unit ground area, while canopy rugosity quantifies the variation in VAI distribution horizontally and vertically (Table 1, Atkins et al. 2018). We collected data along two, 40m transects per plot; one running North-to-South and the other East-to-West. The PCL produces a vegetation hit-grid, mapping a cross-section of vegetation distribution throughout the canopy (Fig. 1). Plot-scale VAI and canopy rugosity were calculated using the fo*restr* R package (Atkins et al. 2018). Details on PCL construction, operation, and other PCL-derived canopy traits can be found in an array of publications (Parker et al 2004, Hardiman & Bohrer et al. 2011, Atkins et. al 2018, Gough et al. 2020).

Root structure was derived from 2-dimensional belowground sampling conducted along the same transects sampled via PCL throughout July 2021 (Fig. 1, Table 1). Roots were collected from four soil depths (0-10cm, 10-20cm, 20-40cm, 40-60cm) using a beveled steel conduit pipe with a 7 cm inside diameter. Collection took place every 10 m along each transect (Figure 1) in two plots per stand ($n = 22$ total) producing a study-wide sample size of 198 vertical cores made up of 792 total increments. Sampling to this depth captures >90% fine roots in our soils (He et al. 2013). Each soil increment was sieved (using 2-mm mesh) for roots, roots were rinsed and oven dried, and then separated into fine (< 2mm diameter) and coarse (> 2mm diameter) pools before weighing. A subsample of roots from each soil depth increment in each stand was ashed in a muffle furnace at 500 °C for 12 hours to adjust for mineral content. We found no statistical difference in mineral content by stand (ANOVA: $F = 0.88$, $p = 0.56$) or depth (ANOVA: $F = 2.34$, $p = 0.09$) and, therefore, applied a standard adjustment of the site-averaged 29% mineral content to all root samples. Fine root density was calculated as grams per cubic centimeter of soil for each increment, averaged by column, then averaged across each plot. We calculated fine root complexity from vertical and horizontal fine root density distributions using the same formula applied to PCL-derived voxelized hit grids in the calculation of canopy rugosity (Hardiman et al. 2017):

$$[1]\ \textit{Fine Root Complexity}=(\sigma\ (\sigma\ [\textit{fine root density}]_{z})_{x})$$

where z is the vertical depth increment, x is the horizontal transect sampling location, and σ is standard deviation.

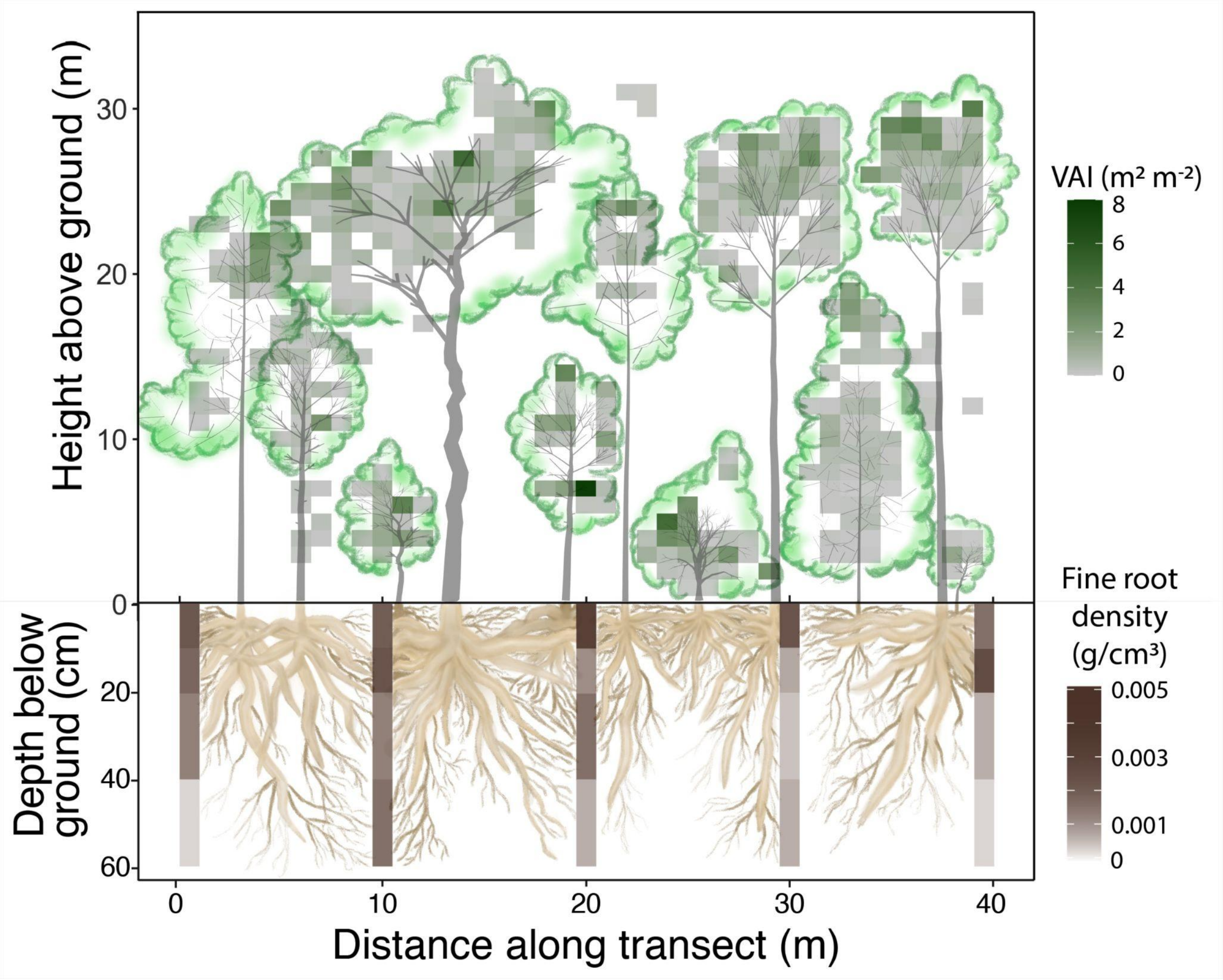

Figure 1. Stylized cross-section of above- and belowground structural relationships, derived from terrestrial lidar and root excavations, respectively. Both approaches provide spatially explicit data on the vertical and horizontal (i.e., 2-dimensional) distribution of canopy or root biomass, permitting the derivation of above- and belowground measures of biomass density and complexity. This example depicts voxelized vegetation area index (VAI) and fine root density along a 40-m transect in the mixed deciduous and needleleaf (MIX) late successional stand. VAI voxels are to scale (1m x 1m) and root density (8-cm wide) voxels are proportionally exaggerated for illustrative clarity. Illustrations by Daulton White.

Table 1. Descriptions and sources of above- and belowground vegetation structure derived from portable below-canopy LiDAR aboveground and root excavations at multiple soil depth increments.

	Category	Unit	Description	References
Aboveground				
Canopy Rugosity	*Complexity*	*m*	*Ratio of VAI variance in horizontal and vertical directions*	Hardiman et al. 2017
Vegetation Area Index (VAI)	*Density*	*dimensionless*	*Mean of column summed vegetation area index*	Atkins et al. 2018
Belowground				
Fine Root Complexity	*Complexity*	*cm*	*Ratio of fine root density variance in horizontal and vertical directions*	This study, modified from Hardiman et al. 2017
Fine Root Density	*Density*	*g/cm^3*	*Mean of column averaged fine root mass per unit soil volume*	Zhou & Shangguan 2007

Soil respiration and microclimate

In situ growing-season soil respiration was collected during the same year as root and canopy structure sampling, on July 26-28, 2021, using a LiCOR-6400 paired with a LI-6400-09 soil CO_2 flux chamber at 5 soil collars per plot. Each collar is a 10cm diameter PVC pipe that sits 3-5cm deep into the soil and was installed in 2014. To account for within-plot spatial variation in respiration rates, collars were arranged 3m, 6m, 9m, 12m, and 15m in a spiral starting northward around and away from plot center. Chamber conditions were set to 400±10ppm CO_2 and two measurements of CO_2 efflux were recorded at each collar and averaged for analysis. Alongside respiration, soil moisture (in the top 20 cm depth) and temperature (in the top 7 cm depth) were collected 2cm away from each collar using a Campbell Hydrosense II and built-in LI-6400 thermocouple, respectively. Analyses were restricted to soil microclimate and respiration collected once in 2021 to align with root and canopy structure characterized in the same growing season; however, all data were comparable to averages from repeated measurements at the same plots during the prior year, 2020(Clay et al. 2022, *in press*).

Light absorption was estimated as the fraction of photosynthetically active radiation (fPAR) absorbed by the canopy. In each plot, fPAR was measured using an AccuPAR LP-80 along the same 40-m perpendicular transects used to sample canopy and root structure. Below canopy PAR observations were taken 1 m above the forest floor approximately every 1 m along the transect, then compared to open-sky, above-canopy reference measurements. All light data were recorded within two hours of solar noon on cloudless days after full leaf-out, in late June or early July 2021.

Statistical analysis by objective (O)

O1: Change in canopy and root structure over succession

To compare changes in above- and belowground structure over succession in chronosequence stands and mean difference among late successional stands, we used linear regression and ANOVA, respectively ($\alpha = 0.05$). For all analyses, plots ($n = 29$ for canopy data, $n = 22$ for root data) were considered the experimental unit, with stand-level means and standard errors presented in corresponding figures. Simple linear regression was used to determine whether changes over time in the cut-only and cut-and-burn chronosequences were significant (α = 0.05) and slope differences assessed by comparing 95% parameter confidence intervals. Late successional stands serve as less-disturbed references, representative of age and community structures that would be common in the absence of stand-replacing disturbances a century ago (Nave et al. 2019); thus, we compared these late successional references with the oldest chronosequence stands via ANOVA with Tukey's HSD pairwise comparisons. Assumptions of linearity, normality, and homogeneity of variance were checked using visual inspection of scatter, Q-Q, and residual plots using the *stats* package in base R (R Core Team 2013).

O2: Bivariate relationships between canopy structure, root structure, and soil microclimate

We used simple linear regression to assess bivariate relationships between canopy rugosity or VAI and soil temperature, soil moisture, fine root density, and fine root complexity (α = 0.05). Assumptions of linearity, normality, and homogeneity of variance were checked using visual inspection of scatter, Q-Q, and residual plots in R.

O3: Multivariate pathways from canopy structure to soil respiration

To provide an integrative assessment of hypothesized biotic and abiotic interactions mediating canopy structure-soil respiration interactions, we compared separate path analyses that included canopy rugosity or VAI as starting explanatory variables. We omitted plots from our analysis for which roots were not collected, leaving a sample size of 22, and log-transformed fine root density data to meet homogeneity of variance requirements. Path analyses were evaluated by confirmatory factor analysis and goodness-of-fit metrics were calculated using the *sem* package in R (Fox 2006). To balance model complexity with our limited sample size, we only retained variables in our path analysis that were significantly correlated with VAI or canopy rugosity in O2. We first ran full models containing all pathways hypothesized *a priori* to link canopy structure and soil respiration, incorporating mechanistically-grounded variables and interactions supported by prior literature, but not previously tested in a multivariate framework (Figure S1).We then reduced models by retaining variables with P < 0.1, applying a slightly more conservative alpha than the conventional default of 0.15 used in multivariate model selection because of our small sample size (Steyerberg et al. 2009). We compared full and reduced models using Akaike information criterion (AIC) and sample-size adjusted Bayesian information criterion (SABIC) scores.

Results

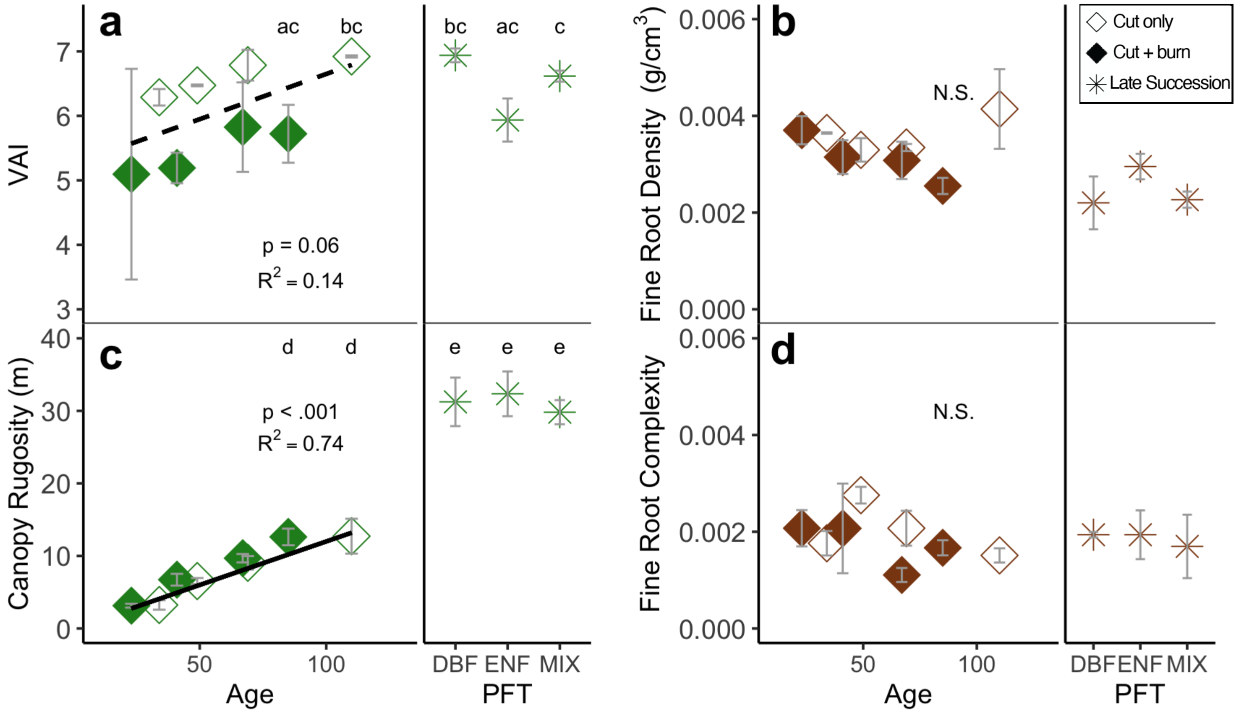

Figure 2. Side-by-side comparisons of above- and belowground measures of vegetation density (a, b) and complexity (c, d) for cut only and cut and burn chronosequences, and late successional stands. The dashed trendline illustrates a common (for both chronosequences) marginally significant (P<0.1) relationship and the solid line a shared significant (P<0.01) relationship. P and adjusted R^2 values are presented for significant linear models. Letters indicate significant

pairwise differences among late successional and the oldest chronosequence stands. Means ± SE., VAI--vegetation area index, N.S.--not significant

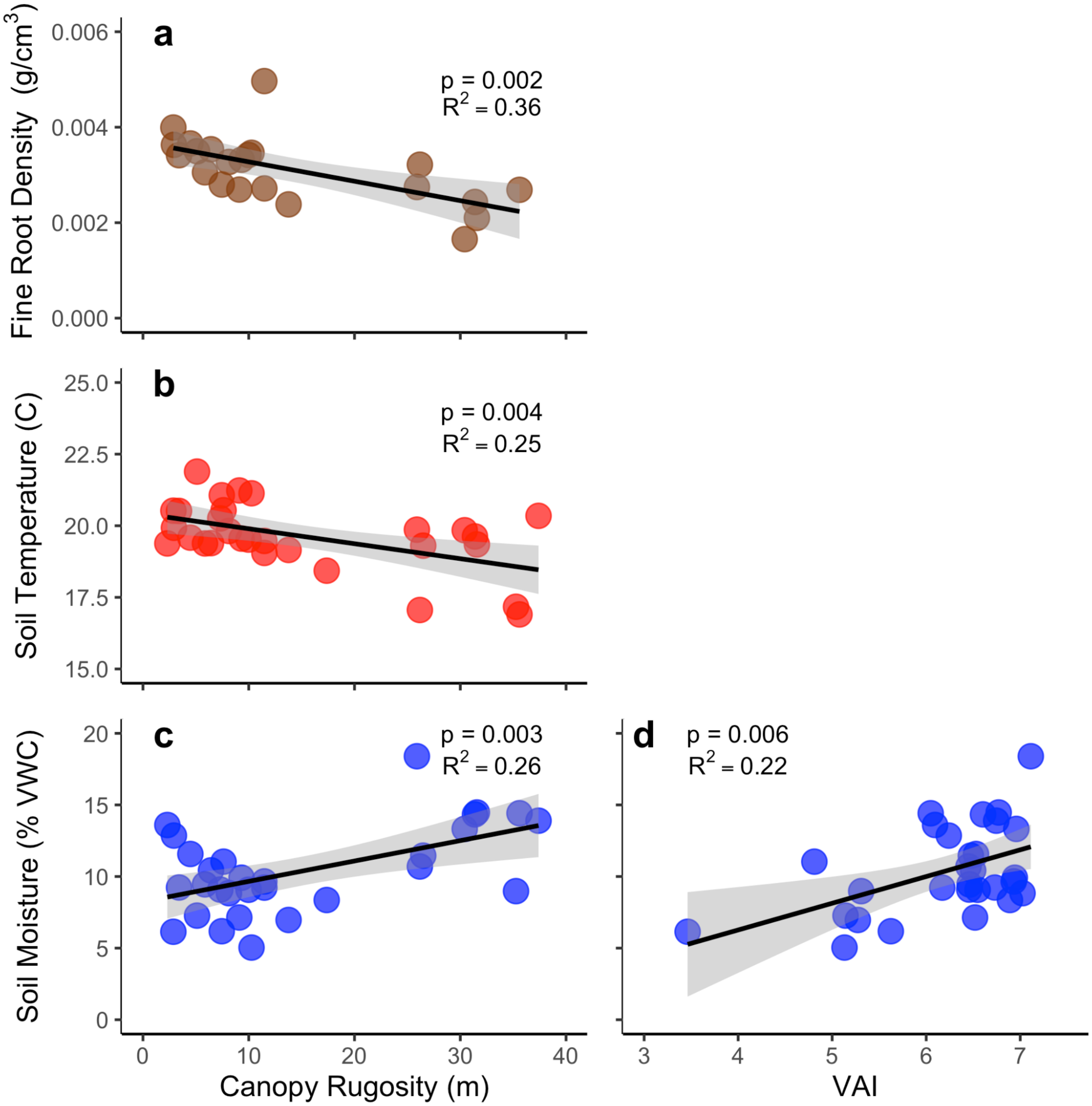

Figure 3. Canopy rugosity in relation to mean plot fine root density (a), soil temperature (b), and soil moisture (c) and vegetation area index (VAI) in relation to soil moisture (d). Solid lines denote significant linear relationships and gray shaded areas illustrate 95% confidence intervals. VWC--volumetric water content. Non-significant (P > 0.05) relationships are not shown.

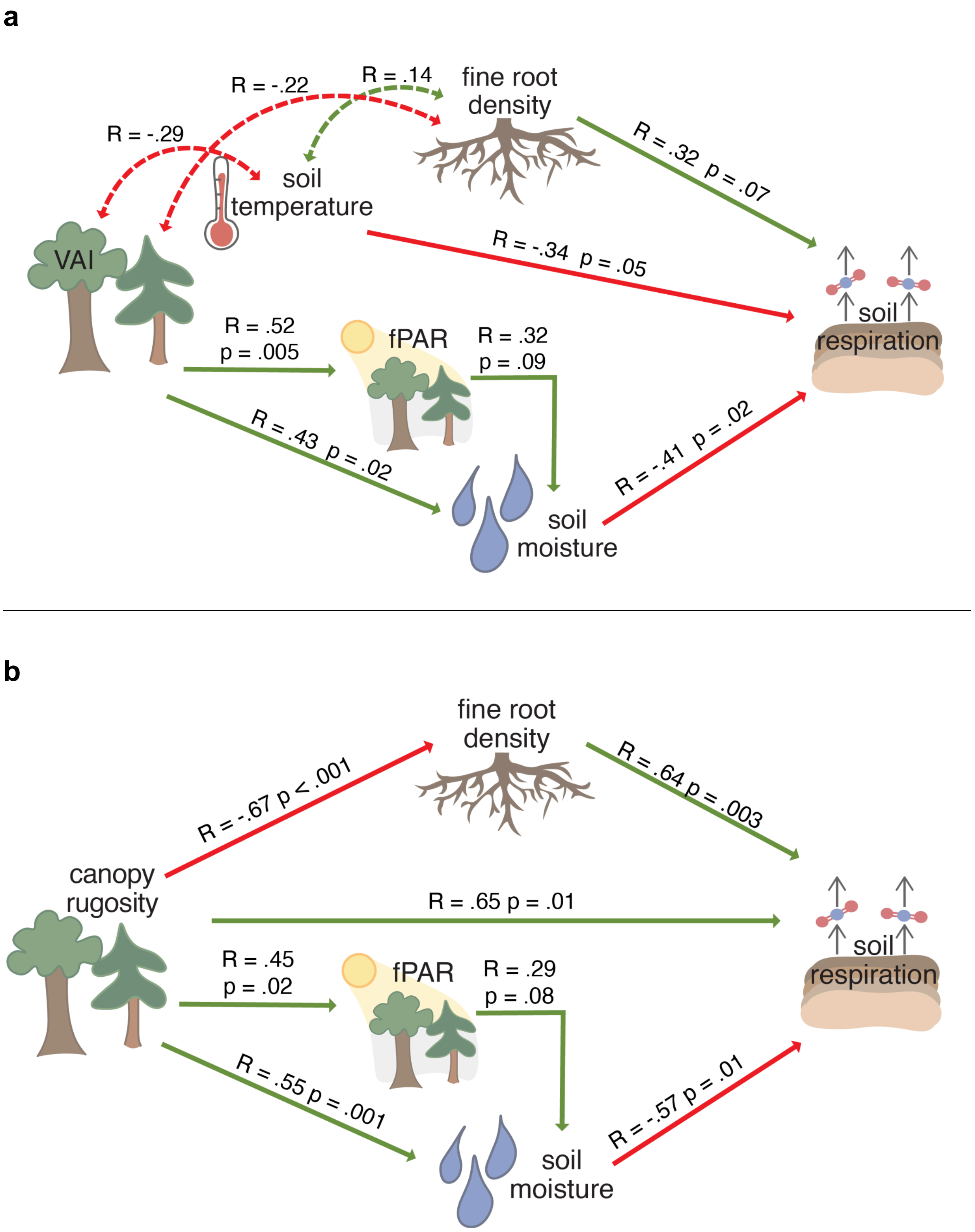

Figure 4. Path diagrams illustrating direct and indirect biotic and abiotic relationships coupling soil respiration to vegetation area index (VAI, a) and canopy rugosity (b). Respective correlation coefficients and p-values for best-fitting VAI (AIC = 104, SABIC = 86) and canopy rugosity

(AIC = 88, SABIC = 66) models presented. Arrow color indicates direction of correlation where positive correlations are green and negative correlations are red. Dashed and curved lines illustrate significant covariance between variables.

Table 2. Summary of model goodness-of-fit statistics for multivariate pathways (illustrated in Figure 4) including chi-square estimation, root mean square error approximation (RMSEA), confirmatory factor index (CFI), standardized root mean square residual (SRMR), Akaike information criterion (AIC) scores, and sample-size adjusted Bayesian information criterion (SABIC) scores. The best-fitting model is highlighted in green.

Canopy trait	**$\chi 2$**		**RMSEA**		**CFI**	**SRMR**	**AIC**	**SABIC**
	Estimate	P-value	Estimate	P-value				
Canopy rugosity reduced model	1.8	0.61	<.001	0.64	1.00	0.04	88	66
VAI reduced model	7.7	0.26	0.11	0.29	0.927	0.12	104	86
Canopy rugosity full model	5.6	0.34	0.08	0.38	0.986	0.07	155	125
VAI full model	6.8	0.23	0.13	0.26	0.922	0.11	178	148

O1: Canopy and root structure over succession

Aboveground structure was more dynamic over successional timescales than belowground structure. Canopy rugosity and VAI increased similarly in the two chronosequences over ~100 years of successional development (Fig. 2), a trend that corroborates prior findings at our site (Scheuermann et al. 2018). There is some evidence (p = 0.06) of increasing VAI from 5.1 to 6.9 with stand age (Fig. 2a). Pairwise comparisons suggest that the VAI of the oldest cut and burn stand was similar that of the ENF stand, the oldest cut only stand was similar to the DBF stand, and the MIX stand was similar to all other stands (Fig. 2a). Canopy rugosity was much more dynamic, increasing four-fold from 3.1 m to 12.7 m over nearly a century (Fig 2b). Late successional stands had higher canopy rugosity than the oldest cut only and cut and burn stands, around 30 m ($p < 0.001$, $F = 13.94$) (Fig 2b).

In contrast to aboveground structure, there were no significant changes over time or among secondary and late successional stands in fine root density or complexity (Fig.2). Fine root density varied from ~0.002 g/cm^3 to ~0.004 g/cm^3 among stands and displayed no significant trend over the course of succession ($p = 0.97$) or difference between oldest chronosequence and late succession stands ($p = 0.47$, $F = 1.26$) (Fig. 2c). Similarly, fine root complexity, averaging 0.002 g/cm^3, exhibited no significant successional trend ($p = 0.20$) or differences between the oldest secondary and late successional stands ($p = 0.49$, $F = 1.19$) (Fig. 2d).

O2: Bivariate relationships between canopy structure, root structure, and soil microclimate

Canopy rugosity was more closely correlated with belowground biotic and abiotic factors than VAI (Fig. 3). Canopy rugosity exhibited negative relationships with fine root density ($p = 0.002$) and soil temperature ($p = 0.004$), and a positive relationship with soil moisture ($p = 0.003$) (Fig. 3). VAI was positively correlated with soil moisture ($p = 0.006$), but not soil temperature ($p = 0.29$) or fine root density ($p = 0.39$) (Fig. 3). Fine root complexity did not correlate with either VAI ($p = 0.49$) or canopy rugosity ($p = 0.73$), and thus was not retained as an explanatory variable for multivariate path analyses.

O3: Multivariate pathways from canopy structure to soil respiration

Path analysis revealed that canopy rugosity was more strongly coupled to soil respiration than VAI through process-mediating biotic and abiotic factors (Fig. 4, Table 2). Canopy rugosity was biotically linked to soil respiration through a direct, positive correlation as well as an indirect pathway mediated by fine root density (Fig. 4b). The fine-root-mediated pathway connects a negative correlation between canopy rugosity and fine root density (Fig 3a.) with a subsequent positive relationship between fine root density and soil respiration (Fig. 4b). Canopy rugosity explained 53% of soil moisture variation through two positively correlated relationships; one direct and one light absorption-mediated (Fig. 4b). These relationships with soil moisture served as an abiotic link from canopy rugosity to soil respiration (Fig. 4b). Although a relationship between canopy rugosity and soil temperature emerged in bivariate analysis (O2, Fig. 3b), soil temperature was not retained as a significant mediator between canopy rugosity and soil respiration in a multivariate context (Fig. 4b). In contrast to the more statistically robust pathways in our reduced canopy rugosity model, VAI did not directly relate to soil respiration and significant covariance existed amongst VAI, soil temperature, and fine root density (Fig. 4a), suggesting that VAI was not as strongly linked to soil respiration at our site through the factors that we examined. Overall, the best canopy rugosity multivariate model incorporating biotic and abiotic factors accounted for 43% of plot-scale variation in soil respiration, while the best VAI model accounted for 35%.

Discussion

Our results demonstrate that forest canopy complexity is an integrator of mechanistically-grounded biotic and abiotic drivers of soil respiration at our site. Canopy rugosity, one of many recently developed metrics of canopy structural complexity (Ehbrecht et al. 2021), strongly predicts the spatio-temporal dynamics of aboveground vegetation and production (Scheuermann et al. 2018, Atkins et al. 2018, Gough et al. 2019). Our findings show that similar predictive capabilities may extend to soil respiration, a more poorly constrained C flux (Bond-Lamberty et al. 2020) through canopy complexity's effects on fine root density, canopy light interception, and soil moisture. If applicable to other sites and ecosystems, our findings provide a mechanistically defensible basis for more broadly inferring stand-level variation in soil respiration from remotely sensed above-ground vegetation complexity.

We found that canopy rugosity was a more robust predictor of soil respiration than VAI, and was more strongly tied to established biotic and abiotic drivers of soil C fluxes. While our analysis is the first to elucidate interconnected biotic and abiotic pathways coupling canopy complexity and soil respiration, assessments of individual pathways offer support for our findings. For example, our analysis shows that canopy complexity is correlated with soil microclimate through its effects on light transmission. Complex forest canopies absorb more light (Ishii et al. 2004, Atkins et al. 2018), limiting the light energy reaching the forest floor and reducing evaporation from soils (Flerchinger and Pierson 1991, Forrester et al. 2012). Taller, denser, and more complex canopies also maintain higher levels of interior humidity and reduce vertical mixing of within-canopy air (Renaud et al. 2011, Von Arx et al. 2012), which further reduces evaporative losses from soil. In our analysis, the abiotic pathways connecting VAI and canopy complexity to soil respiration were similar, but stronger relationships with canopy rugosity mirror other studies that suggest measures of canopy complexity are more closely related to canopy light absorption than aboveground vegetation area metrics (Atkins et al. 2018, Gough et al. 2019).

Spatio-temporal patterns of above- and belowground vegetation structure at our site are similar to those of other forests; however, we did not find evidence for stand-scale mirroring of above- and belowground structures. Our results reinforce findings that the successional development of canopy structure is more dynamic than that of root structure (Cavelier, Estevez, and Arjona

1996). However, a complete lack of spatial correspondence between above- and belowground structure is at odds with some prior observations demonstrating correlations between root and canopy gaps (Ostertag 1998, Taskinen et al. 2003, Suchewaboripont et al. 2015, Hardiman et al. 2017). A successional divergence of above and belowground measures of vegetation density and complexity suggests vertical fine root densities and distributions are conserved over time, and consequently decoupled from canopy complexity. In our closed-canopy forest stands, more conserved fine root structures may reflect the relatively fixed vertical taper and depth of rooting observed in temperate forests (Zhou & Shangguan 2007), with most fine roots contained within the top 20 cm of mineral soil in all of our stands. In contrast, the aboveground vegetation height, which is highly variable among forests worldwide (REF), places a primary constraint on canopy complexity because taller canopies contain more space within which to construct heterogeneous vegetation arrangements (Gough et al. 2021). Although not structural analogs, we did find that fine root density decreased as canopy rugosity increased (Fig. 3), possibly because canopy complexity is positively correlated with site productivity (Gough et al. 2019) and more productive forests invest relatively less in root production (Nadelhoffer et al. 2000, Litton et al. 2007), which could in turn result in lower autotrophic respiration (Litton et al. 2007). Interpreting canopy rugosity-root density relationships in the context of our multivariate path analysis, fewer fine roots in complex and productive forests appear to be an ecologically plausible mediating biotic pathway connecting canopy rugosity (but not VAI) to soil respiration.

While many of the pathways we found connecting canopy structure to soil respiration are supported by prior ecological observations, interactions between these pathways are less straightforward. For example, other studies from our site show that more complex stands have equal or lower soil respiration rates than less complex stands (Liebman et al. 2017, Clay et al. 2022 *in press*), which is counter to the direct, positive relationship between canopy complexity and soil respiration in our multivariate analysis. In this context, the direct pathway that we observed from canopy rugosity to soil respiration could be caused by complexity's positive influence on C fixation and production (Hardiman et al. 2011, Gough et al. 2019), factors that limit stand-scale soil respiration (Bond-Lamberty et al. 2004). More complex canopies sequester more carbon (Hickey et al. 2019, Gough et al. 2019) and, in doing so, may allocate proportionally more photosynthate to belowground metabolism (Hogberg et al. 2001). This pathway could also indicate the effect of additional direct and/or indirect variables, such as soil texture and soil nutrient status, which also vary across the study landscape and are not mutually exclusive to other variables and pathways (Hofmeister et al. 2019; Nave et al. 2017; 2019). Spatial variation of soil nutrients in particular is associated with canopy complexity-soil respiration relationships in systems with similar vegetation types elsewhere (Suchewaboripont et al. 2015). Future inclusion of additional canopy structure-soil respiration mediating factors may further resolve the drivers connecting canopy complexity and soil respiration, while increasing the overall predictive power of our multivariate path model.

In application, our findings linking canopy rugosity to soil respiration suggest that an immense and poorly constrained C flux could be inferred from the ground-to-spaceborne remote sensing of aboveground vegetation structure. There is a longstanding history of using remotely sensed vegetation indices to model respiration (Xiao et al. 2018), with recognition that canopy structural features serve as proxies for biotic drivers of C fluxes (Reichstein et al. 2003). Our results extend such findings, suggesting that newer lidar-derived canopy complexity measures may better integrate and encompass biotic *and* abiotic drivers of respiration. If such relationships apply to landscapes beyond the one we have studied here, then canopy complexity, rather than conventional indices of vegetation area and canopy reflectance, may be more robust predictors of spatial variation in soil respiration and, consequently, useful to constraining ecosystem and earth system models (REF). Such large spatial-scale assessments of canopy complexity-soil respiration interactions are now feasible using open data provided by lidar-equipped aircraft and satellites (Beland et al. 2019, Shiklomanov et al. 2020, Gough et al. 2022) and ecological networks, including FLUXNET (Baldocchi et al. 2001).

Limitations and future directions

We recognize that this study is limited by site specificity and small sample size. Our limited sample size captured a large range of canopy variability (Fig. 2), plant functional types, and disturbance histories relevant to temperate forests, but does not explicitly address influences of variables such as soil texture or nutrient availability on respiration. Additionally, this study did not address the influence of species diversity on forest structure, rooting strategies, or soil moisture patterns that may be relevant to belowground C processes. Despite these limitations, related work at UMBS establishing PCL-derived canopy structure-primary production relationships (Hardiman et al. 2011) has been further explored and confirmed in temperate forests across the Eastern United States (Atkins et al. 2018, Gough et al. 2019). Extending our analysis of canopy structure-soil respiration interactions to larger spatial scales is an important next step in determining if our findings apply at broader scales.

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Supplementary Documents

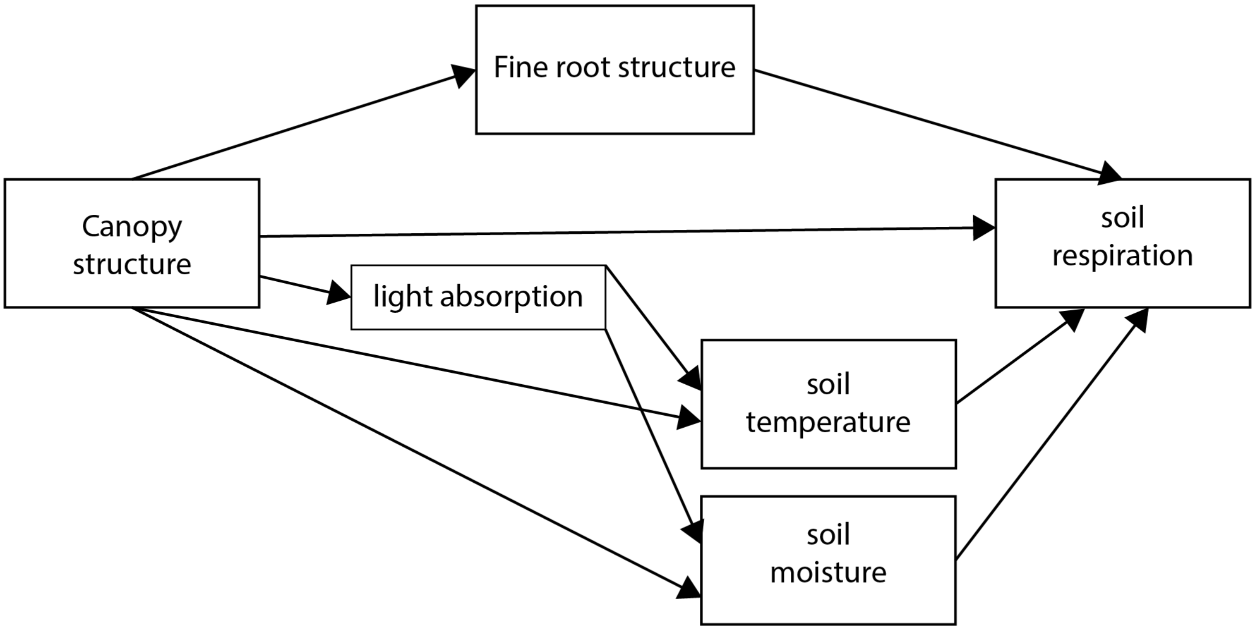

Figure S1. Pathways linking canopy structure to soil respiration hypothesized *a priori* to our experiment. Paths are based on variables that tie canopy structure to aboveground carbon cycling (i.e. light absorption) or drive soil respiration (i.e. soil temperature, soil moisture, and fine roots) in order to ground our hypotheses in well-established mechanistic relationships.