Soil Respiration and Related Abiotic and Remotely Sensed Variables in Different Overstories and Understories in a High Elevation Southern Appalachian Forest.

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Abstract

Accurately predicting soil respiration (R_s) has received considerable attention recently due to its importance as a significant carbon flux back to the atmosphere. Even small changes in R_s can have a significant impact on the net ecosystem productivity of forests. Variations in R_s have been related to both spatial and temporal variation due to changes in both abiotic and biotic factors. This study focused on soil temperature and moisture and changes in the species composition of the overstory and understory and how these variables impact R_s. Sample plots consisted of four vegetation types: eastern hemlock (*Tsuga canadensis* L. Carriere) dominated overstory, mountain laurel (*Kalmia latifolia* L.) dominated understory, hardwood dominated overstory, and cinnamon fern (*Osmundastrum cinnamomeum* (L.) C.Presl) dominated understory with four replications of each. Remotely-sensed data collected for each plot, light detection and ranging, and hyperspectral data, were compiled from the national ecological observatory network (NEON) to determine if it could improve predictions of R_s. Soil temperature and soil moisture explained 76% of the variation in R_s. There were no statistically significant differences between the average annual R_s rates among the vegetation types. However, when looking at monthly R_s, cinnamon fern plots had statistically higher rates in the summer when it was abundant and hemlock had significantly higher rates in the dormant months. At the same soil temperature, the vegetation types' R_s rates were not statistically different. However, the cinnamon fern plots showed the most sensitivity to soil moisture

changes and were the wettest sites. NDLI was the only vegetation index (VI) to vary between the vegetation types. It also correlated with R_s for the months of August and September. PRI, NDVI, and NDNI also correlated with September's R_s. In the future, further research into the accuracy and the spatial scale of VIs could provide us with more information on the capability of VIs to estimate R_s on a broad scale. The differences we found in monthly R_s rates among the vegetation types might have be driven by varying litter quality and quantity, litter decomposition rates, and root respiration rates. Future efforts to understand carbon dynamics on a broader scale should consider the temporal and finer-scale differences we observed.

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Abstract (public)

Forests have the ability to sequester carbon from our atmosphere. Soil respiration (R_s) plays a role in a forest's ability to do so as it is a significant source of carbon dioxide back to the atmosphere. Therefore, understanding the process of R_s under varying conditions is gaining more attention. As of now we have a relatively good understanding of R_s under managed forest ecosystems such as pine plantations. This particular study examined R_s under different overstories and understories in a high elevation Southern Appalachian forest in order to get a better understanding of R_s under a natural hardwood system. The four vegetation types under consideration were an eastern hemlock (*Tsuga canadensis* L. Carriere) dominated overstory, a hardwood overstory with little to no understory, a mountain laurel (*Kalmia latifolia* L.) dominated understory, and a cinnamon fern (*Osmundastrum cinnamomeum* (L.) C.Presl) dominated understory. Differing temporal variations of R_s were observed under the vegetation types. We found monthly differences in rates among vegetation type however, an overall annual difference in R_s rates between vegetation types was not observed. This simply indicates the importance of observing R_s under different time scales to get a better understanding of its variation. We also calculated vegetation indices from remotely-sensed data to explore any relationships to R_s as well as if the indices themselves could improve out model. A vegetation index is a number that is calculated for every pixel in a remotely sensed image and represents plant vigor or abundance. Few significant relationships were found between the indices

and R_s. Future work may want to better understand vegetation indices' spatial extent and accuracy in order to find whether they may be beneficial in R_s estimation. Understanding the influence of varying vegetation type and soil temperature and moisture on R_s will ultimately improve our ability to predict what drives changes in carbon fluxes.

Rachel Lynn Hammer

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Table of Contents

List of Tables

List of Figures

Introduction

Forests play a critical role in the global carbon cycle. In the northern hemisphere alone, forests sequester about $7x10^8$ metric tons of carbon annually (Gough et al. 2008). Forested ecosystems capture carbon dioxide (CO_2) through photosynthesis and lose CO_2 through respiration which includes soil respiration (R_s). R_s is a significant carbon flux back to the atmosphere and even small changes can have significant impacts on CO_2 concentrations in the atmosphere. Therefore, forests are being investigated extensively as they likely will play a large role in the management of global CO_2 emissions (Schlesinger and Andrews, 2000). In fact, U.S. forested land offsets about 12% of the annual greenhouse gas emissions (Murray et al. 2005). In Virginia alone, 15.8 million acres or about 62% of the land is forested, a substantial amount of land for carbon sequestration (VDOF, 2014). Recent research has suggested that if the globe was forested to its potential, forests could reduce the atmospheric carbon pool by 25% (Bastin et al. 2019). Soil respiration (R_s) makes up 30-80% of total ecosystem respiration. Variations in R_s have been identified both spatially and temporally by researchers and these variations are affected by both biotic and abiotic factors (Wang et al., 2015). The biotic factors can include canopy cover, leaf area, and litter deposits. Biotic and abiotic factors can directly influence each other and often interact. Soil moisture and soil temperature are considered the two most influential abiotic factors influencing soil respiration (Liu et al., 2014). Soil moisture and soil temperatures' effect on R_s varies depending on climate and soil conditions. For example, in one case study, R_s decreased due to low soil water content in a savanna landscape under dry conditions, but in a tropical swamp forest, R_s decreased as the depth to ground water decreased. Among different ecosystems, the study found soil temperature sensitivity of R_s per 10˚ C temperature change at a global scale to be between 1.43 to 2.03 (Adachi et al. 2017). In most studies investigating both soil temperature and moisture, soil temperature has the most influence on R_s when soil moisture is not limiting or in excess (Yu et al., 2011).

This study will examine both abiotic and biotic factors for relationships with R_s in a biologically diverse high elevation southern Appalachian forest. The abiotic factors being examined are soil temperature and soil moisture while the biotic factors are changes in the understory and overstory composition. Vegetation effects the soil microclimate, the quantity and quality of litter deposited, as well as the rate of root respiration (Akburak and Makineci, 2013). One study in particular found R_s rates under a soybean field to be 520 g C m^{-2} while it averaged 720 g C m^{-2} under a switchgrass field. They found lower R_s rates and higher soil temperatures under the soybean fields suggesting that another element was overriding the soil temperature's impact. They found this factor to be soil moisture. Uncultivated switchgrass tends to have denser living and dead plant matter cover and thus can hold more moisture in the soil (Raich and Tufekcioglu, 1999). This is one example of the many ways vegetation can alter soil microclimate ultimately affecting the R_s rates. I hope to explain how discrete changes in the overstory and understory impact R_s and provide more information on how different species in Southern Appalachian forests affect carbon sequestration. Studies have not examined in depth the comparison of R_s across overstories and understories in Appalachian forests. In particular, the high elevation Appalachian forests are biologically very complex and thus understanding how they cycle carbon under the smaller spatial scales could help us scale up to the landscape.

In 1974, the National Academy of Sciences challenged the forestry and remote sensing sectors to come together, observe, and overcome any obstacles in their way of integrating (Bergen et al., 2000). Even today foresters and remote sensing specialists still run into problems, mainly with maximizing the efficiency of using existing remotely-sensed data. Remote sensing is most often utilized by a forestry agency or company in operational procedures, special projects studying specific forest features, and by research in academic settings or for a forest agency or company. In these areas of study, it is not practical nor economically feasible to gather all the data needed by field measurements. Remotely-sensed data can build upon existing data sets to fill in gaps in the landscape. Costs of remotely-sensed data were once a problem; however, costs for Landsat 7 and 8 data have gone down making the cost per unit area less of an issue. Also, over the years we have learned that we cannot see remote sensing as a stand-alone solution instead it should be viewed with other geospatial data in order to get the best result. Currently, remote sensing's applications in forestry cover a wide range of subjects including terrain analyses, forest management, updating forest inventories, forest cover type delineation, and mapping burned areas (Bergen et al., 2000). I will be focusing on its application in estimating R_s.

Relating R_s to easily obtained remotely-sensed variables has several advantages. It is cheaper, allows estimations of R_s at regional, continental, and global scales, and can be used to view remote locations (Lees et al. 2018). Vegetation reflectance can give us insight into plant phenology by examining the variation in near-infrared and visible bands. This, in turn, can explain temporal changes in plant photosynthesis associated with the fraction of vegetation cover and vegetation status. Also, we can determine canopy photosynthesis from vegetation indices (VIs) derived from more than one band of reflectance, and since they have a possible relationship with the substrate supply to R_s we can gain more insight into the R_s of the area (Huang and Niu, 2013). Examples of indices include the normalized difference vegetation index (NDVI) and enhanced vegetation index (EVI). NDVI involves looking at the red visible band which vegetation strongly absorbs and the near infrared band which vegetation strongly reflects in a remote sensed image. The calculation is as follows:

(near-infrared band – red band)/ (near-infrared band + red band)

The green vegetation will have higher values for NDVI then browning or unhealthy vegetation. The EVI is similarly calculated but improves on the NDVI by having the ability to detect differences in densely vegetated areas and by also correcting for atmospheric affects. By gaining information on plant photosynthesis and canopy cover we can then estimate CO_2 flux which has been found to correlate with R_s (Huang et al., 2012).

For this study, we will be using Light Detection and Ranging (LiDAR) data which uses a laser to measure variable distance to the earth's surface. It is a form of active remote sensing which means it sends its own pulse of energy down to earth from a sensor then reads energy that is reflected back from the target object. This information can provide three-dimensional information about the shape of the earth and its surface characteristics. Tree canopy levels can be detected. The laser will reach the top of the canopy and reflect some energy back but some of the pulse will penetrate to the other levels and reflect energy back. These are called multiple returns. Then, metrics related to different forest characteristics can be calculated. This includes a closer look into the makeup of the understory as well as the canopy (US Department of Commerce 2012). We will simply be calculating canopy height form LiDAR. We will also examine hyperspectral data which collects information across the electromagnetic spectrum for each pixel in the image. It differs from multispectral imaging by the number and narrowness of the bands. Hyperspectral imaging can consist of hundreds to thousands of bands and are usually 10-20nm while multispectral imaging may deal with about 3-10 bands at a much wider scale (Lees et al. 2018). I will examine if the amount of variability in the R_s data set gathered from the ground is explained by the remotely-sensed data collected over our study area.

Along with soil temperature and moisture, we predict that the vegetation type will have a significant effect on R_s. Our assumption is that the presences of evergreen species, such as the eastern hemlock (*Tsuga canadensis* L. Carriere) dominated overstory and the mountain laurel (*Kalmia latifolia* L.) dominated understory vegetation types will have higher R_s rates in the dormant seasons while the hardwood dominated overstory and the cinnamon fern dominated understory will have higher CO_2 flux in the growing season. We also expect the hardwood dominated vegetation type with little understory will have higher soil temperatures than the others due to a lack of understory that can cool temperatures.

Our specific objectives for this study are as follows:

1. To characterize how the different dominant understory or overstory plant communities affect total R_s and the relationship of R_s to soil temperature and moisture.
2. Determine if remotely-sensed data can predict R_s directly or improve on models using land -based measures of soil temperature and moisture.

General Methods

Site and Stand Description

The study took place on the Mountain Lake Biological Station (Giles Co., VA.), owned and operated by the University of Virginia, as well as near the site of the National Ecological Observatory Network (NEON) tower (Figure 1). The area can be considered a Southern Appalachian northern red oak forest based on the natural communities of Virginia: Classification of ecological groups and community types by the Virginia Department of Conservation and Recreation (Fleming and Patterson, 2017). The distinction of species within this ecological group includes deciduous holly (*Ilex montana* Torrey and A. Gray), white oak (*Quercus alba* L.), black cherry (*Prunus serotina* Ehrh.) striped maple (*Acer pensylvanicum* L.), with occasional small patches of pitch pine (*Pinus rigida* Mill.) and eastern hemlock (*Tsuga canadensis* L. Carriere). The topography across our specific study area can be described as generally flat terrain. The average annual rainfall is 96.5 cm and the average annual snowfall is 66 cm per year. The high temperature in July is 28.9 C˚ and January's low temperature is -5 C˚ (*Climate* 2018). The soil is mapped as a combination of Lily-Bailegap complex (Fine-loamy, siliceous, semiactive, mesic Typic Hapludults) described as very stony and on 2 to 15% slope, as well as generic Fluvaquents described as poorly drained, and on nearly level surfaces (Soil Survey Staff, accessed December 12, 2018). As a result, over relatively short distances, abrupt changes in overstory and understory vegetation occurs. We worked in four distinct vegetation types. Type 1 (hardwood) were areas dominated by an overstory of hardwood species (*Quercus* spp., *Acer* spp.) with little to no understory. Type 2 (cinnamon fern) was a similar overstory but with a heavy understory of cinnamon fern (*Osmundastrum cinnamomeum* (L.) C.Presl). Type 3 (mountain laurel) was a similar hardwood overstory but with a heavy mountain laurel (*Kalmia latifoli*a L.) understory. Type 4 (hemlock) were areas dominated by an eastern hemlock overstory. Four replications of each of the different vegetation types were identified in the landscape which resulted in a total of sixteen plots.

Methods for Objective 1: *To characterize how the different dominant understory or overstory plant communities affect the total R_s and the relationship of R_s to soil temperature and moisture.*

In a 1/50th ha circular fixed radius plot all trees and shrubs greater than 2 cm diameter at 1.37 feet above the ground were identified and measured for diameter in September of 2018. We waited until February of 2019, for the absence of leaves, to collect tree heights to obtain better accuracy using a TruPulse 200 (Laser Technology, Inc., Centennial, CO). In February, about 3% of the trees measured in September were snapped or broken by an ice storm. In order to obtain an estimate of their previous heights before the storm we created a simple linear regression based on the height and DBH data already gathered for undamaged trees of that species. Basal area, trees per acre, relative average dominance (basal area of a given species expressed as a percentage of the total basal area of all species present) and volume per hectare for all the species' in each plot were calculated. Volume was estimated using the equations from Clark and Schroeder (1986). Species-specific equations were used for the following species: blackgum (*Nyssa sylvatica* Marshall.), red maple (*Acer rubrum* L.), black oak (*Quercus velutina* Lam.), northern red oak (*Quercus rubra* L.), and white oak (*Quercus alba* L.). We used either a hard hardwood or soft hardwood equation for the other hardwood species' in the plots. For example, for striped maple (*Acer pensylvanicum* L.), witch-hazel (*Hamamelis virginiana* L.), mountain laurel, and cucumber (*Magnolia acuminata* L.) we used the soft hardwood equation, and the hard hardwood equation for American chestnut (*Castanea dentata* (Marsh.) Borkh.), downy serviceberry (*Amelanchier arborea* (F.Michx.) Fernald), deciduous holly (*Ilex decidua* Walter), and black cherry (*Prunus serotina* Ehrh.). We used species-specific equations for our two conifer species, hemlock and eastern white pine (*Pinus strobus* L.) from Hahn (1984). Biomass of herbaceous understory plants such as ferns were measured using a 1m^2 clip plot in each 1/50th ha plot collected on August 14th 2018 and later dried and weighed. The clip plot was taken 1m distance from the plot center aimed towards the first tree that we measured for DBH. Soil was sampled (0 to 10 cm) at three randomly selected locations in each 1/50th ha plot using a soil push tube. The subsamples were well mixed on site and the composite sample was used for analysis. The 16 soil samples were sent to the Virginia Tech Soil Testing Laboratory to be tested. The nutrients available for plant uptake (P, K, Ca, Mg, Zn, Mn, Cu, Fe, B, and Al) were extracted from the soil with a Mehlich 1 solution using a 1:5 vol: vol soil to extractant ratio and then were analyzed by an inductively coupled plasma atomic emission spectrometer (ICP). The acidity of the soil was determined using an automated pH analyzer. The weight loss on ignition method was used to determine soil organic matter. CEC of the soil was determined by summation of the acidity, Ca, Mg, and K (Maguire and Heckendorn, 2010). We also tested for C:N ratio of the soil using a vario MAX CNS analyzer (Elementar Americas Inc., Ronkonkoma, NY).

In each of the vegetation types, we measured R_s, and soil temperature and moisture in each plot monthly, spanning a full year, in order to get a wide range of soil temperatures and plant phenologies. Three random subsamples were collected in each plot. A Li-Cor 8100-103 gas analyzer (LI-COR Inc., Lincoln, NE, USA) with a survey chamber diameter of 20 cm (soil area of 317.8 cm^2) was used to measure R_s. We placed it directly over the soil surface and applied pressure to ensure a seal. We made sure to not capture any green vegetation in the survey chamber. In the same subsample locations during each visit, a digital thermometer, the AcuRite 00641, (Chaney Instrument Co., Lake Geneva, WI) was used to measure soil temperature at a depth of 12 cm. A hydrosense II soil - water sensor (Campbell Scientific USA, Logan, UT) was used to measure percent volumetric soil moisture from 0-11cm.

Methods for Objective 2*:* *Determine if remotely-sensed data can predict R_s directly or improve on models using land-based measures of soil temperature and moisture.*

The National Ecological Observatory Network (NEON) uses an air observation platform which is an array of instruments mounted on a light aircraft to gather data. The instruments include a hyperspectral imaging spectrometer, a full waveform and discrete return LiDAR, and a high-resolution red, blue, and green (RGB) camera. The hyperspectral imaging spectrometer aboard NEON's aircraft captures 426 narrow spectral bands from 380nm to 2510nm with a spectral sampling of 5nm (Neonscience.org, 2018). We retrieved hyperspectral and LiDAR data of our plots from NEON for the year 2015. We used a Trimble Geo 7x handheld GPS (Trimble Inc., Sunnyvale, CA) to find accurate plot centers. We then converted the GPS data points, taken from the field to a shape file for viewing in ArcGIS (Esri, Redlands, CA, USA). We extracted data from my plot areas. The image analysis software ENVI (Harris Geospatial Solutions Inc., Broomfield, CO, USA) was used to calculate various VIs for the plot areas including Normalized Difference Vegetation Index (NDVI), Enhanced Vegetation Index (EVI), Atmospherically Resistant Vegetation Index (ARVI), Photochemical Reflectance Index (PRI), Normalized Difference Nitrogen Index (NDNI), and Normalized Difference Lignin Index (NDLI). NDVI quantifies vegetation by measuring the difference between near-infrared and red light. EVI improves upon NDVI by including factors in its equation to correct for particles in the air as well as ground cover below the vegetation. ARVI also improves upon NDVI by minimizing the effects of atmospheric scattering (Gonzalez Del Castillo et al, 2018). PRI is used for assessing photosynthetic efficiency, plant stress, and productivity and is sensitive to changes in carotenoid pigments in foliage. NDNI can give us an idea of the amount of nitrogen in vegetation canopies while NDLI can estimate relative amounts of lignin in canopies (Serrano et al, 2002). We examined the differences between the VIs' averages among the vegetation types. We then examined the VIs and the annual average R_s for each plot, the average growing season (May to September) R_s of each plot, the average R_s of each plot for each date sampled, and the average dormant season (October to April) R_s for correlation. We found the average canopy height of each of the sixteen plots from LiDAR data and then examined the relationship of these values to the overall annual average R_s for each plot, the average growing season R_s, average dormant season R_s, and the average R_s of each plot for each date sampled. We incorporated the vegetation indices individually into our base model and observed any changes statistically to assess if they improved the predictability of R_s.

Statistical Analyses

The statistical software JMP ® 14 (SAS Institute, Cary, NC, USA) was used for all analyses. The experiment was performed as a randomized complete block design with four replications. The experimental units were the treatment plots per vegetation type and the average of any subsamples collected in each plot. For R_s, we measured one set of each vegetation type at a time and used time as the block variable in order to remove variation based on daily temporal differences. Variables were transformed as needed in order to meet assumptions of analysis of variance and regression. Vegetation treatment characteristics such as biomass (volume, basal area, and DBH), annual means of R_s, soil temperature, and soil chemistry parameters were analyzed using analysis of variance. Statistical exploration of the variables was performed including scatterplots and determining Pearson's correlation coefficient to look at the relationship of the variables and R_s as well as the relationships between the variables themselves. ukey's HSD Post Hoc test was used to compare the vegetation indices, soil chemistry values, R_s, soil moisture, and soil temperature between the vegetation types. Regression analysis was used to measure the relationships between R_s, soil temperature, soil moisture, soil chemistry parameters, and remotely- sensed variables. Parameter estimates of the vegetation type-specific models were examined for any significant differences using the indicator parameterization estimates table.

Results

Site Characterization

The overstory species' basal area in the cinnamon fern plots were primarily red maple, white oak, and northern red oak (Figure 2a). Basal area in the hardwood plots were similarly dominated by northern red oak, and white oak (Figure 2b). In the hemlock plots 32.0% was white oak, followed closely by hemlock (28.1%) (Figure 2c). Basal area in the mountain laurel plots were primarily dominated by northern red oak (Figure 2d); however, when observing dominance by stems per hectare (Figure 3) we found the plots were dominated by mountain laurel (42.9%) (Figure 3d). Based on stems per hectare the hemlock plots were dominated by hemlock stems (23.9%) (Figure 3c). Characterization by volume (m^3/ha) was similar to the basal area for all the plots (Figure 4 a-d).

Understory clip plot data clearly demonstrated that fern plots were dominated by cinnamon fern with 27.4 kg/ha of vegetation of which 99% were ferns. Hardwood and mountain laurel plots both had only 1.9 kg/ha of herbaceous vegetation respectively. The hemlock plots had nearly no understory vegetation (Table 2).

Our soil C:N ratios were highest under the hemlock (23.3±0.431) vegetation plots followed by the mountain laurel (22.1±0.472), hardwood (21.0±0.700), and lastly the cinnamon fern (20.0±0.451) plots (Table 3a). The C:N ratio for the hemlock plots was found to be statistically higher than the hardwood and cinnamon fern plots based on the Tukey's HSD Post Hoc test. Percent potassium saturation was the only other soil chemical property to significantly differ between the vegetation types based on Tukey's HSD Post Hoc test (Table 3a, b). The hardwood plots had significantly higher percent potassium saturation than hemlock, meaning a higher relative number of CEC sites were occupied by potassium. This can give us an indication for any gross nutrient imbalance (Maguire and Heckendorn, 2010).

Soil Temperature, Soil Moisture, Vegetation Type, and R_s

Simple linear regression indicated a strong positive relationship between soil temperature and R_s (R^2=0.75, P<0.0001) while a weak negative relationship between soil moisture and R_s was found (R^2=0.06, P<0.0001). On average, the cinnamon fern vegetation type had statistically higher soil moisture than the other three vegetation types (Table 4). On average, for all seasons, both soil temperature and R_s were not significantly different across all four vegetation types.

General R_s Patterns

R_s was higher in the growing season and lower in the cooler, dormant months across all vegetation types. No strong patterns between vegetation types were evident. However, April, August, December, and February had R_s values that differed significantly between two or more of the vegetation types. R_s was highest in the cinnamon fern plots during the warmer months. Hemlock plots had higher rates in the cooler months (Figure 5).

R_s in our data did not follow assumptions of normality therefore a natural logarithmic transformation was used on R_s. The model showed unequal variance. We took the square root of soil temperature and the natural log of soil moisture to resolve this issue. Soil temperature alone, explained 75% of the variation observed. R_s was best explained by a model that included soil moisture, and soil temperature (R^2=0.76). There was no significant interaction between soil temperature and soil moisture. Stand characterization such as basal area, volume, and trees per ha, as well as soil chemical properties did not add any significance to our model.

When comparing the parameter estimates of our vegetation-type specific models we found significant differences (Table 7). Holding certain parameters to an average for the growing season allowed us to examine how each of the vegetation type models responded to soil temperature (Figure 6) and soil moisture (Figure 7) separately. The soil moisture slope is significantly more negative for the cinnamon fern plots than the hemlock or mountain laurel plots. For every unit increase in soil moisture, the cinnamon fern plots' R_s lowers at a faster rate than both the hemlock and mountain laurel R_s rates. The intercept in the cinnamon fern model was significantly higher than the mountain laurel model's intercept. The soil temperature slope values did not significantly differ between the vegetation types models (Figure 6).

Remotely-sensed variables and R_s

VIs

With the exception of NDLI, none of the VIs differed between vegetation types (Table 9). NDLI was greatest for the mountain laurel vegetation type. The annual average R_s value, the average growing season R_s, the average non-growing season R_s, and all VIs were not significantly correlated. When we examined the correlation between the VIs and the average R_s by date sampled we found some statistically significant relationships (Table

9). Our R_s under each of the sixteen plots for the sample date of August showed a significant correlation (P=0.0214, R^2=0.324) to the NDLI. For the R_s for the sample date of September we found a correlation between several VIs including NDLI (P=0.0462, R^2=0.25), NDVI (P=0.02, R^2=0.329), PRI (P=0.0285, R^2= 0.30), and NDNI (P=0.025, R^2=0.31). For all significant correlations the first cinnamon fern plot acted as a high leverage data point. When removed the relationship between August's R_s and NDLI was stronger (R^2=0.64), however all other significant correlations drastically decreased in strength when the high leverage value was removed. We found no reason to remove the value as the number was still an accurate representation of the site. Accounting for soil temperature and soil moisture variation, adding our vegetation indices individually into our base model did not explain any additional variation of R_s.

Mean Canopy Height

We found no significant relationship between mean canopy height calculated from LiDAR data and average growing season R_s. We also, found no significant relationship between average nongrowing season R_s and mean canopy height. Mean canopy height correlated with the September sample date (P=0.0254, R^2=0.30).

Discussion

Soil temperature explained 75% of the variation in our model, alone. Many other studies have found a similar influence by soil temperature. A study by Yu et al. (2011) in a 50-year-old oriental arborvitae (*Platycladus orientalis* (L.) Franco) plantation in China found soil temperature to explain the variation in R_s by 82% in the overall annual cycle. It was the main determinant for R_s when soil moisture was not limiting. This is just one of many studies including Bilal et al. (2017), and Templeton et al. (2011) that found soil temperature to be the strongest driver of R_s. Inclán et al. (2007) reported that soil temperature was the main driver of R_s variation unless soil moisture reached levels below 15% which it then became the better predictor.

The annual average soil temperature did not differ significantly between vegetation types. When looking by sampling date, we found soil temperature to be statistically lower in the hemlock plots over the growing season (Table 5). The denser, darker canopy of hemlocks may shade the soil resulting in cooler soil temperatures in the summer. In terms of soil moisture, we found cinnamon fern to have a higher average soil moisture value than all other vegetation types. The difference could simply be due to the fact that cinnamon fern requires moist environments in order to reproduce. They are usually found on poorly drained low ground, in thickets, wet marshy woods, swamps, ditches, and streambanks (Walsh R.A., 1994).

We know R_s varies both spatially and temporally. Temporal variation of R_s includes diurnal, weekly, seasonal, or annual changes (Lai et al, 2012). When we examined R_s seasonally we found significant differences among the vegetation types. The cinnamon fern's R_s was significantly higher in the month of August most likely due to its sheer abundance at that time of the year. We expected to see significantly higher R_s rates for hemlock on our cooler sampling dates than the other vegetation types which is the case for our study. As an evergreen, when there are mild winter days, hemlocks continue photosynthesizing and in some cases with approximately the same photosynthetic capacity as in the summer (Burkle and Logan, 2003). We know R_s is influenced by substrate availability and thus strongly linked to photosynthesis, litterfall, and plant metabolism and, therefore higher activity aboveground in evergreens in the cooler months can lead to higher activity belowground (Ryan and Law, 2005). Seasonality effected R_s differently across different vegetation types for a study by Lai et al (2012). R_s for winter wheat, *E. angustifolia* shelterbelt, *T. ramosissima* scrubland, and *H. ammondendron* + *R. soongorica* scrubland was highest in July and then decreased with temperature while *P. communis* grassland peaked in September and declined in October. Overall, *E. angustifolia* had the greatest seasonal variation in R_s. We found seasonal differences between R_s rates; however, unlike the study mentioned, the average annual R_s rates did not significantly differ between vegetation types. Though we found differences in our soil moisture levels with cinnamon fern having the wetter sites, that did not provide a big enough influence on R_s rates to observe any differences between sites. Soil moisture usually explains less of the variation in R_s than soil temperature. Our results were not unlike the study by Akburak and Makineci (2013). They explored the temporal changes of soil respiration under different tree species. They found monthly R_s to be statistically different between the tree types, however there was no significant difference between the mean annual R_s rates among the tree types. R_s followed their soil moisture trend. Despite no significant difference for the annual mean R_s between vegetation types, we still encountered monthly differences. We would need to explore the root respiration rates, quality and quantity of litter, and more soil characteristics to see if perhaps these monthly differences could be due to the influence of vegetation type perhaps as a secondary effect.

When comparing our vegetation-type specific models, there were statistically significant differences in the response of R_s to soil moisture (Table 8). Cinnamon fern's model for predicting R_s was the most sensitive to soil moisture changes (Figure 7). The response of R_s to soil temperature between all of the vegetation types did not differ (Figure 6). We found few differences in the soil chemical properties between the vegetation types. Perhaps the soil environment was similar enough across the vegetation types that the vegetation's effect on annual mean R_s was minimal. It's important to note that the spatial variation in R_s is usually driven by soil characteristics as well as biological processes while temporal variation is usually driven by climatic variables (Qi and Xu, 2001). For cinnamon fern, perhaps its high R_s rates in August and its lower R_s rates in the dormant months even out, resulting in an average annual R_s rate similar to that of the hemlock that can continually photosynthesize throughout the year. A study by Martin and Bolstad (2005), examined R_s and its influences such as moisture and site characteristics under five different forest types. They noted an apparent lack of effect of dominant vegetation type on R_s. Similar to our study, the site conditions were relatively homogenous. Our topography did not significantly differ, nor did site characteristics, or soil chemical properties. In our case, vegetation type may have minimal utility in predicting R_s on a spatial scale. A continuation of Martin and Bolstad's (2009) previous study examined more forest types including clear-cuts, ash elm, aspen, northern hardwood, wetland edges, and red pine forests. Variability in R_s was observed both within forest type and between forest type. This study included more site variability including differences in topography. They noted an interrelatedness of topographically induced hydrologic patterns and soil chemistry unlike in their previous studies where topography was the same across all forest types (Martin et al., 2009). Bilal e al. (2017) compared R_s across cover types in a southern Appalachian hardwood forest. Their model included soil temperature and soil moisture as the two drivers of variation. When comparing R_s rates between our study and theirs, we found that our R_s rates were almost double that of Bilal et al (2017) at the same soil temperature. At about a soil temperature of 10˚C, their R_s rates were about 2 μmol CO_2 m^{-2} s^{-1} whereas our R_s rates at that temperature were 4 μmol CO_2 m^{-2} s^{-1}. Their sites were located on either foot or shoulder slopes and at lower elevation than ours. They found that the differences in site quality correlated with the differences they saw in R_s between the cover types. Perhaps similar to the study by Martin et al. (2009), they experienced topographically induced soil moisture regimes which may have limited R_s compared to our sites. In fact, our sites were wetter, overall. The average soil moisture values at our sites were higher than their highest soil moisture value. However, after using our growing season average soil moisture value (33%) in Bilal's prediction model we found only a slight increase in R_s rates. The is a good indicator that differences between our sites are not due to soil moisture differences as it only explains very little variation in R_s.

There has been controversy in the past with some studies saying the influence of vegetation on soil microclimate is sufficient enough to explain differences in R_s among vegetation types (Raich and Tufekcioglu, 2000). Others say the correlation between climate characteristics, net primary productivity, and R_s has caused scientists to speculate which factors are truly driving the differences in R_s between different vegetation types (Raich and Schlesinger, 1992). In a study by Reichstein et al (2003), they corrected the R_s data for soil temperature and moisture influences resulting in site-specific, standardized respiration rates. These standardized rates were correlated with leaf area index and leaf production indicating that both climate and vegetation type played important roles in explaining the spatial variability in R_s. On the contrary, another study found a correlation between net primary productivity and R_s when comparing various ecosystem types. They believed the correlation was mainly caused by a background correlation of both factors with climate variables (Raich and Schlesinger, 1992). In the future, we should focus on developing models that try to isolate vegetation effects in order to get a better picture of species influence on R_s.

The use of remotely-sensed VIs to predict R_s

We found some patterns between our remotely-sensed indices and R_s. When examining the relationship between the indices and R_s by sampling date, we found significance. Both August and September had significant relationships with the NDLI. NDLI was negatively correlated with R_s for these months. The negative correlation may be related to the fact that litter with higher lignin content is more difficult to break down by heterotrophic organisms, lowering R_s (Chapin et al., 2017). The reason we may have found correlations with the R_s rates for September and August may simply be because that time of the year has high R_s as seen in Figure 5. Overall, most of the correlations we did find between R_s and the six different VIs were relatively weak (Figure 9). Seasonally, it may be difficult to correlate R_s with greenness VIs due to soil temperature or moisture limitation which may limit the influence of substrate supply from photosynthesis to R_s (Irvine and Law, 2002).

Remotely-sensed VIs, in the past, have been more closely correlated with gross primary production (GPP) than with respiration components (Ling-Hao et al., 2002). For example, Wylie et al (2003) looked at estimating daytime and nighttime carbon flux using an integrated NDVI (iNDVI). Linear relationships between the iNDVI and daytime carbon flux were strong with R^2 values of 0.72 to 0.92 for the three years observed. The iNDVI even correlated better with their nighttime carbon flux (R^2=0.34) than NDVI correlated with our values of R_s (R^2=0.07) in our study. It is important to note that NDVI tends to saturate at high vegetation densities and can be sensitive to background reflectance (Huete et al, 2002). Our sites, therefore, could have been too highly vegetated for the use of NDVI or there was little change in GPP across our vegetation types.

VIs can be estimators of changes in biophysical parameters such as green leaf area index (GLAI), and canopy chlorophyll content (Chl_{canopy}). GLAI and Chl_{canopy} in turn, can explain most of the variation in R_s much like in the study by Huang et al (2012). Huang et al. (2012) saw the potential to investigate how VIs may directly relate to R_s due to the strong relationship between VIs and biophysical parameters. They found strong correlations between daily mean R_s and EVI as well as daily mean R_s and $CI_{red\ edge}$. The study was looking at maize and winter wheat fields and the indices were calculated from hyperspectral reflectance data taken by a portable spectroradiometer. The differences in land use types between our study and the one mentioned above as well as the difference in capturing the hyperspectral data, may explain the differences we saw in the ability of VIs to estimate R_s. In fact, the study mentioned that sites with evergreen species might not exhibit as drastic seasonal change in vegetation greenness as crop fields which may make the estimation of R_s by VIs more difficult.

Wang et al (2004) used canopy temperature measured from an infrared thermometer in addition to soil surface temperature to estimate R_s. Interestingly, they found that the model with the canopy temperature derived from infrared technology performed better than the model solely looking at soil surface temperature to estimate R_s. Though this experiment was limited to explaining R_s on a small spatial scale, expanding our remote sensing tools to more than just VIs for the estimation of R_s may still be beneficial to understanding overall carbon cycling in an ecosystem.

In our study, it's important to consider the difference between the spatial scale of the data collected on site versus the spatial scale of the remotely-sensed indices. In turn, this could have explained the few weak relationships we found between the two. A study by Hogrefe et al. (2017), was exploring the use of NDVI as an estimator of biomass and nutritional value of forage plants in Alaska. They wanted to explore the differences in NDVIs ability as an estimator when it was collected in two different spatial scales, from moderate resolution satellite data (250 x 250 m) and from a handheld spectrometer (20 cm diameter area). The NDVI derived from the handheld spectrometer performed better in the model ($R^2 \geq 0.67$) than the NDVI from the satellite data ($R^2 < 0.40$). This study showed the importance of considering scale in modeling in order to increase accuracy in predictions. Remotely-sensed variables from satellite data may do a better job predicting factors such as GPP, R_s, or biomass on a broader spatial scale where there may be more obvious variation in these factors. Many processes may appear homogenous at a local scale but as the observation scale becomes larger may start to appear heterogenous. Therefore, processes may seem important at one scale but remain trivial in another scale (Wu and Li, 2009). Further research into the accuracy and the spatial scale of VIs could provide us with the information needed to know whether they will have the ability to predict R_s on the landscape scale in the future.

Conclusion

In agreement with many studies, most of the variation in R_s was explained by soil temperature. The significant differences in R_s we saw among the vegetation types were observed by sampling date not for the annual mean. This suggests the importance of looking at R_s under various temporal scales including weekly, monthly, and yearly in order to get a better understanding of variation. Conducting the study over a number of years may provide us with more insight into vegetation's influence on R_s. The vegetation may still have driven the differences we saw in monthly R_s by their influence on litter decomposition rates, soil microbiology, and other soil microclimate characteristics. Future studies may want to gather more data on litter differences, partition the R_s into its autotrophic and heterotrophic components, and take the LAI under each vegetation type.

The ability of remotely-sensed VIs to predict R_s was limited in our study. NDLI was the only VI to show a difference between vegetation types as well as correlate with certain monthly R_s rates. The lack of relationships we found otherwise may have been due to a scale discrepancy between our ground data and the VIs calculated from satellite data. Studies may want to focus on examining VIs ability to predict processes under broader spatial scales as well as get a better understanding of VIs spatial accuracy.

In conclusion, R_s was mainly driven by soil temperature and moisture. That being said, our model may provide the basis for future models estimating R_s under natural hardwood systems in the Appalachian Mountains.

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Table 1. Stand characteristics for hardwood, cinnamon fern, hemlock, and mountain laurel vegetation types; diameter at breast height (cm), trees per ha, basal area (m^2/ha), and volume (m^3/ha).

Vegetation Type	Diameter at Breast Height (cm)	Trees per ha	Basal Area m^2/ha	Volume m^3/ha
Hardwood	10.0±1.5	1525±174	25.7±4.2	296±37
Cinnamon Fern	12.4±4.2	900±250	20.8±3.6	217±51
Hemlock	18.0±1.7	1150±134	45.4±5.3	441±53
Mountain Laurel	12.0±1.3	1613±168	35.8±7.5	390±94

Table 2. Weight (kg/ha) of herbaceous understory and fraction of the weight that is fern versus non fern for hardwood, cinnamon fern, hemlock, and mountain laurel vegetation types.

Vegetation Type	Total Weight (kg/ha)	Fraction Fern	Fraction Nonfern
Hardwood	1.9	0.01	0.99
Cinnamon Fern	27.4	0.99	0.01
Hemlock	0.32	0.06	0.94
Mountain Laurel	1.9	0	1

Table 3a. Average soil chemical parameters for the hardwood, cinnamon fern, hemlock, and mountain laurel vegetation types A different letter signifies a significant difference ($P < 0.05$) between vegetation types using Tukey's HSD Post Hoc test.

Vegetation Type	C:N ratio	pH	BpH	Extractable P ppm	Extractable K ppm	Extractable Ca ppm	Extractable Mg ppm	Extractable Zn ppm	Extractable Mn ppm
Cinnamon Fern	20.0±0.45 B	4.0±0.04	4.9±0.08	5.3±2	45.3±2.3	56.5±7.4	22.8±2.8	1.9±0.2	5.1±2
Hemlock	23.3±0.43 A	4.1±0.1	4.9±0.08	4.5±3	36.5±0.65	62.5±6.7	19.8±1.4	1.3±0.1	4.6±0.8
Mountain Laurel	22.1±0.47 AB	4.0±0.08	5.0±0.06	1.8±0.5	44.0±2.6	65.3±7.9	21.5±2.8	1.3±0.2	4.8±0.6
Hardwood	21.0±0.70 B	4.3±0.09	5.2±0.08	2.3±0.3	43.3±3.5	47.5±1.0	17.5±1.2	1.3±0.05	5.0±1

Table 3b. Average soil chemical parameters for the hardwood, cinnamon fern, hemlock, and mountain laurel vegetation types continued.

Vegetation Type	Extractable Cu ppm	Extractable Fe ppm	Extractable B ppm	% OM	CEC meq/100g
Cinnamon Fern	0.5±0.05	76.3±4.2	0.1±0	7.0±0.6	9.5±0.5
Hemlock	0.3±0.05	92.3±13	0.1±0	8.7±0.5	9.3±0.5
Mountain Laurel	0.4±0.03	95.2±6.0	0.1±0	7.6±0.8	9.0±0.4
Hardwood	0.4±0.03	102.4±7.68	0.1±0	7.7±0.9	7.7±0.5

Table 3c. Average soil chemical parameters for the hardwood, cinnamon fern, hemlock, and mountain laurel vegetation types continued. A different letter signifies a significant difference (P < 0.05) between vegetation types using Tukey's HSD Post Hoc test.

Vegetation Type	% Acidity	% Base saturation	% Ca saturation	% Mg saturation	% K saturation
Cinnamon Fern	93.9±0.71	6.2±0.7	3.0±0.4	2.0±0.3	1.3±0.1 AB
Hemlock	93.9±0.49	6.2±0.5	3.4±0.4	1.8±0.1	1.0±0.07 B
Mountain Laurel	93.2±0.62	6.8±0.6	3.6±0.4	1.9±0.2	1.3±0.05 AB
Hardwood	93.7±0.17	6.3±0.2	3.1±0.2	1.8±0.05	1.4±0.06 A

Table 4. Average soil respiration (R_s), average soil temperature (at 6 and 12 cm), and average soil moisture (0 to 11 cm) and standard errors across all the sampling dates for hardwood, cinnamon fern, hemlock, and mountain laurel vegetation types. A different letter signifies a significant difference (P < 0.05) between vegetation types using Tukey's HSD Post Hoc test.

Vegetation Type	R_s (μmol CO_2 m^{-2} s^{-1})	Soil Temperature at 12 cm (C˚)	Soil Moisture at 0 to 11 cm
Hemlock	5.99 ± 0.36	10.51 ± 0.504	33.83 ± 0.606 B
Cinnamon Fern	6.19 ± 0.45	11.01 ± 0.526	36.52 ± 0.503 A
Mountain Laurel	5.55 ± 0.34	11.02 ± 0.543	33.32 ± 0.489 B
Hardwood	5.43 ± 0.35	11.07 ± 0.542	33.27 ± 0.468 B

Table 5. Average soil temperature (˚C) at 12cm for hardwood, cinnamon fern, hemlock, and mountain laurel by sampling month. A different letter signifies a significant difference ($P < 0.05$) between vegetation types using Tukey's HSD Post Hoc test.

Vegetation Type	April	May	June	July	August	September	October	December	February
Hardwood	6.11±0.04A	11.4±0.16A	14.7±0.10A	16.0±0.10A	17.1±0.06A	17.8±0.11A	11.1±0.20	3.17±0.14 B	2.37±0.06
Cinnamon Fern	5.64±0.10 B	10.7±0.10 B	14.5±0.08A	15.8±0.12A	16.7±0.10 B	17.5±0.09 B	12.1±0.18	3.61±0.15AB	2.60±0.10
Hemlock	5.57±0.06 B	10.0±0.09 C	13.8±0.08 B	15.1±0.07 B	16.3±0.05 C	16.9±0.04 C	10.6±0.84	3.78±0.13A	2.68±0.10
Mountain Laurel	5.73±0.04 B	10.9±0.09 B	14.8±0.06A	16.1±0.05A	17.1±0.07A	17.6±0.07AB	11.4±0.10	3.20±0.15AB	2.38±0.09

Table 6. Multiple linear regression parameter estimates for soil respiration (R_s) in the form: Ln (R_s) = Intercept + A [sqrt (Soil Temperature)] + B [Ln (Volumetric Soil Moisture)] for the vegetation type-specific models for hardwood, cinnamon fern, hemlock, and mountain laurel (n=108).

Model	Intercept	A	B	RMSE	R^2	R^2 adjusted
General	-0.3679	0.8595	-0.2627	0.47	0.75	0.75
Hardwood	0.1811	0.8083	-0.3957	0.51	0.72	0.71
Cinnamon Fern	1.9222	0.9145	-0.9480	0.47	0.79	0.78
Hemlock	-0.5659	0.8240	-0.1403	0.42	0.77	0.76
Mountain Laurel	-1.9419	0.8829	0.1527	0.44	0.79	0.79

Table 7. P-values for the comparison of parameter estimates of the vegetation-type specific models. Starred values are statistically significant at the α=0.05 level.

Vegetation Type Comparisons	Intercept	A	B
Hardwood-vs-Cinnamon Fern	0.2644	0.1264	0.1874
Hardwood-vs-Hemlock	0.5679	0.8227	0.4701
Hardwood-vs-Mountain Laurel	0.1449	0.2662	0.1692
Cinnamon Fern-vs-Hemlock	0.0710	0.2084	0.0266*
Cinnamon Fern-vs-Mountain Laurel	0.0111*	0.6468	0.0071*
Hemlock-vs-Mountain Laurel	0.2737	0.3977	0.3888

Table 8. Average values and standard errors of the VIs: normalized difference vegetation index (NDVI), enhanced vegetation index (EVI), atmospherically resistant vegetation index (ARVI), photochemical reflectance index (PRI), normalized difference lignin index (NDLI), normalized difference nitrogen index (NDNI), and canopy height (m) for hardwood, cinnamon fern, hemlock, and mountain laurel vegetation types. A different letter signifies a significant difference between vegetation types.

Vegetation Type	NDVI	EVI	ARVI	PRI	NDLI	NDNI	Canopy Height (m)
Cinnamon Fern	0.91±0.003	2.1±0.014	0.87±0.002	-0.04±0.003	-0.01544±0.0003 B	-0.05±0.002	16.7±1.498
Hardwood	0.91±0.002	2.1±0.013	0.86±0.004	-0.04±0.001	-0.01446±0.0002 AB	-0.05±0.001	17.2±1.110
Hemlock	0.91±0.002	2.1±0.011	0.87±0.003	-0.04±0.002	-0.01464±0.0002 AB	-0.05±0.001	17.8±0.403
Mountain Laurel	0.90±0.001	2.1±0.008	0.86±0.003	-0.04±0.001	-0.01437±0.0002 A	-0.05±0.001	18.2±0.257

Table 9. P-values and R^2 values for the VIs and R_s sampling dates that were found to have significant correlations.

Sampling Date	**Vegetation Index**	**P-value**	R^2
August (238)	NDLI	0.0214	0.32
September (273)	PRI	0.0285	0.3
September (273)	NDVI	0.02	0.33
September (273)	NDLI	0.0462	0.25
September (273)	NDNI	0.025	0.31

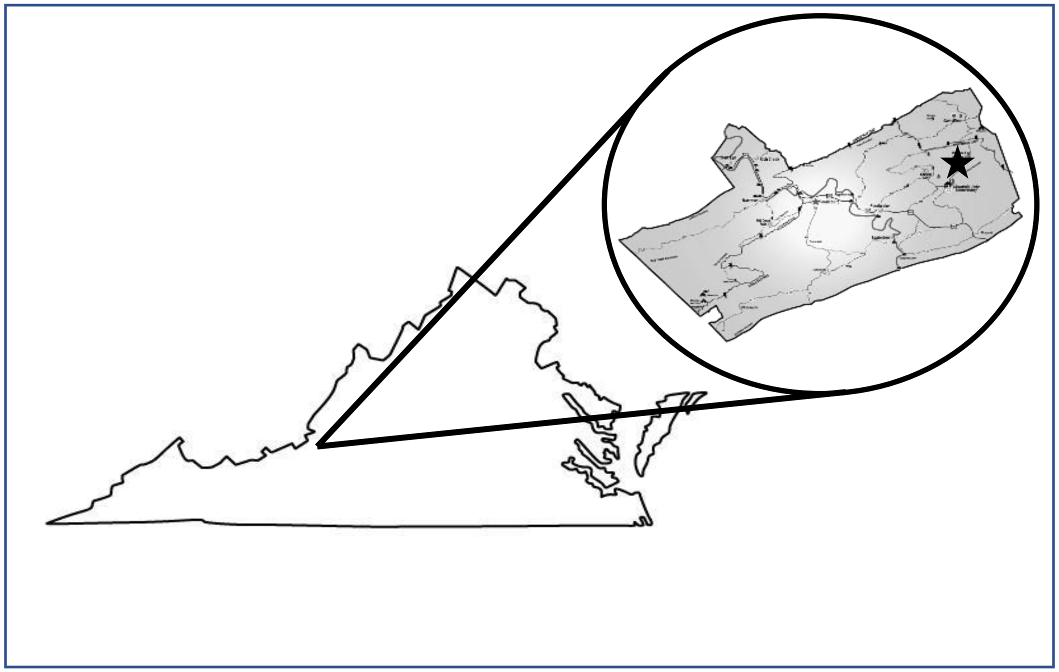

Figure 1. Study site location in Giles County at Mountain Lake Biological Station and at the site of the National Ecological Observatory Network tower.

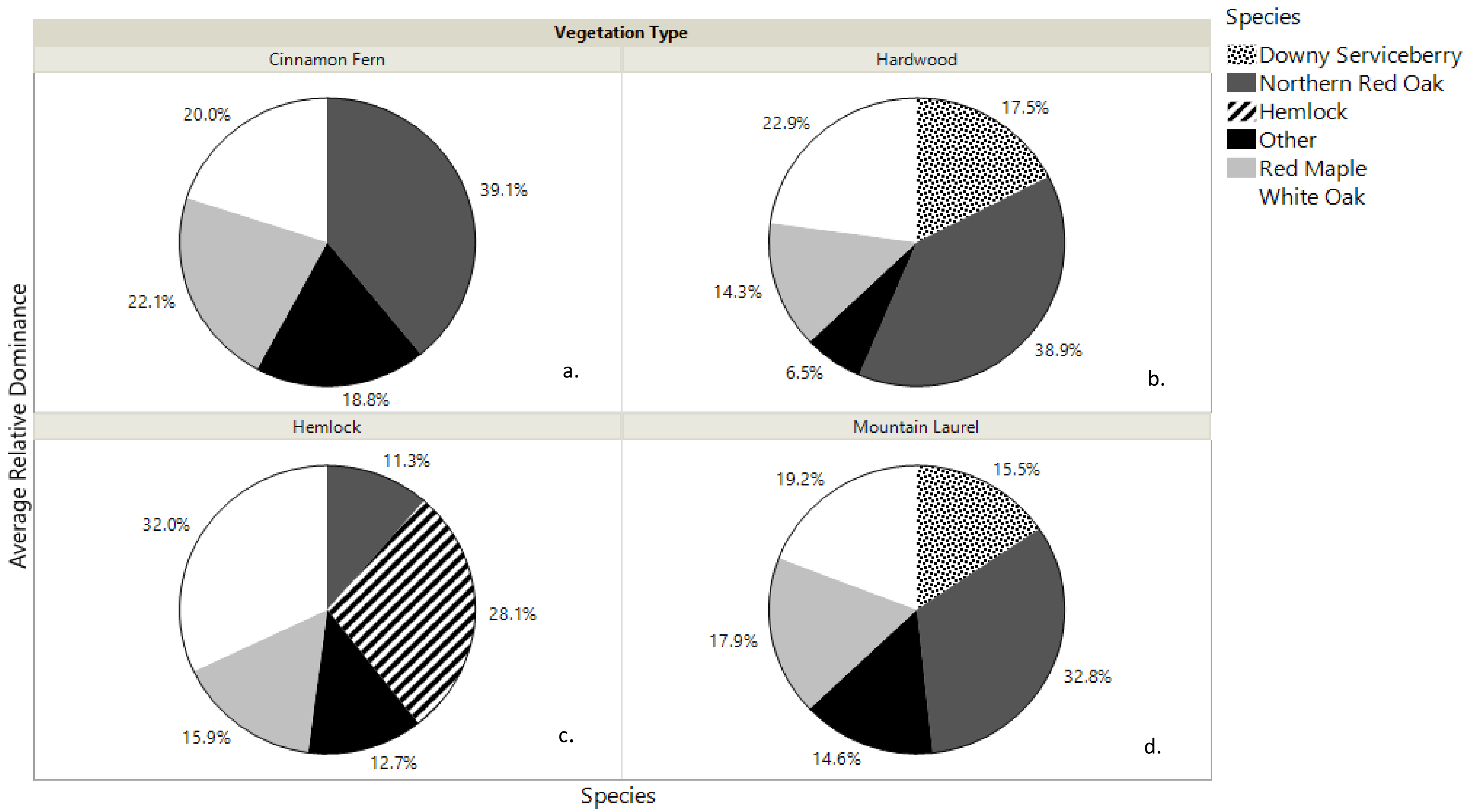

Figure 2a-d. Average species composition based on basal area (m^2/ha) for hardwood, cinnamon fern, hemlock, and mountain laurel vegetation types.

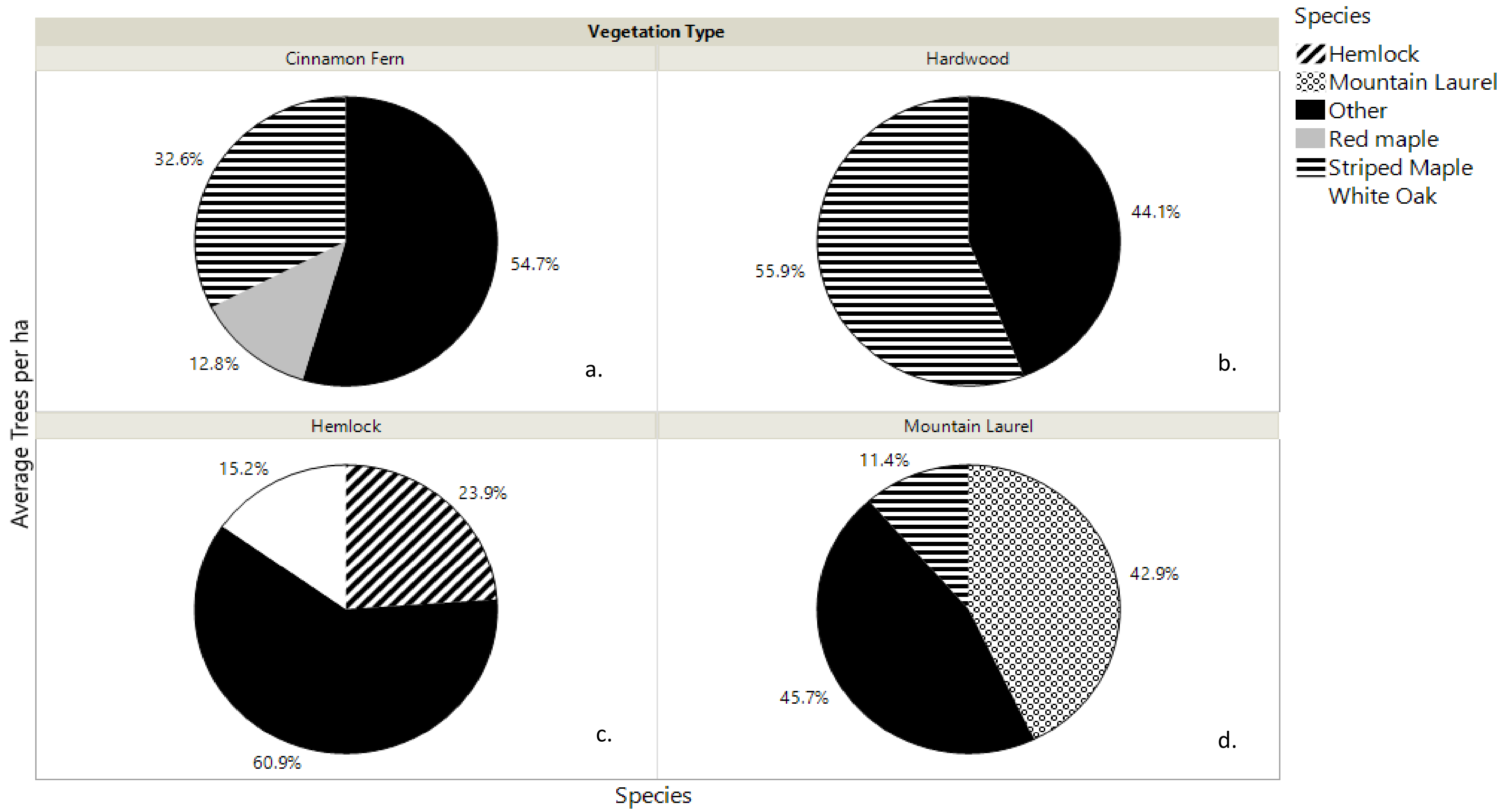

Figure 3a-d. Average species composition based on trees per ha for hardwood, cinnamon fern, hemlock, and mountain laurel vegetation types.

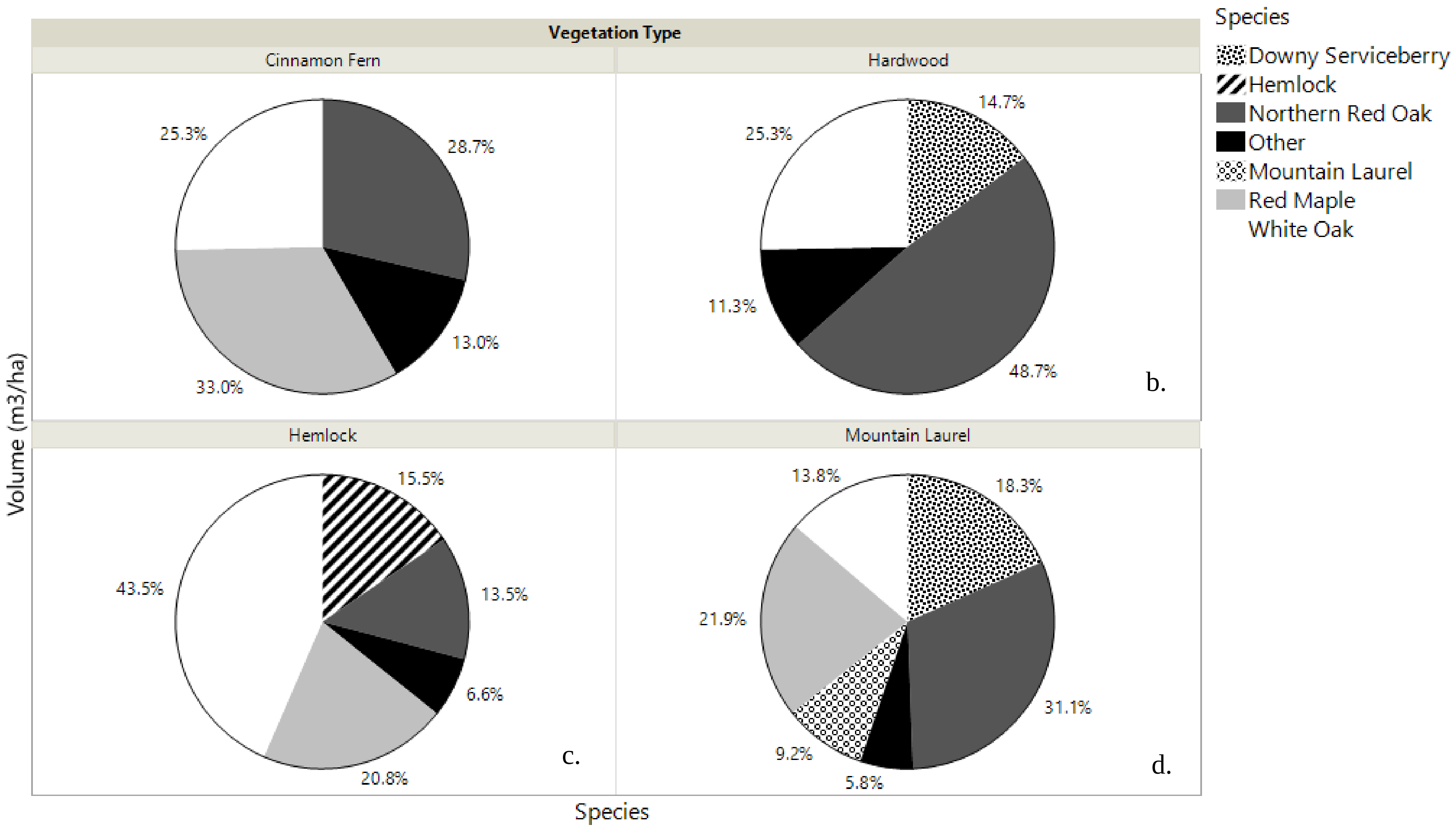

Figure 4a-d. Average species composition based on volume (m^3/ha) for hardwood, cinnamon fern, hemlock, and mountain laurel vegetation types.

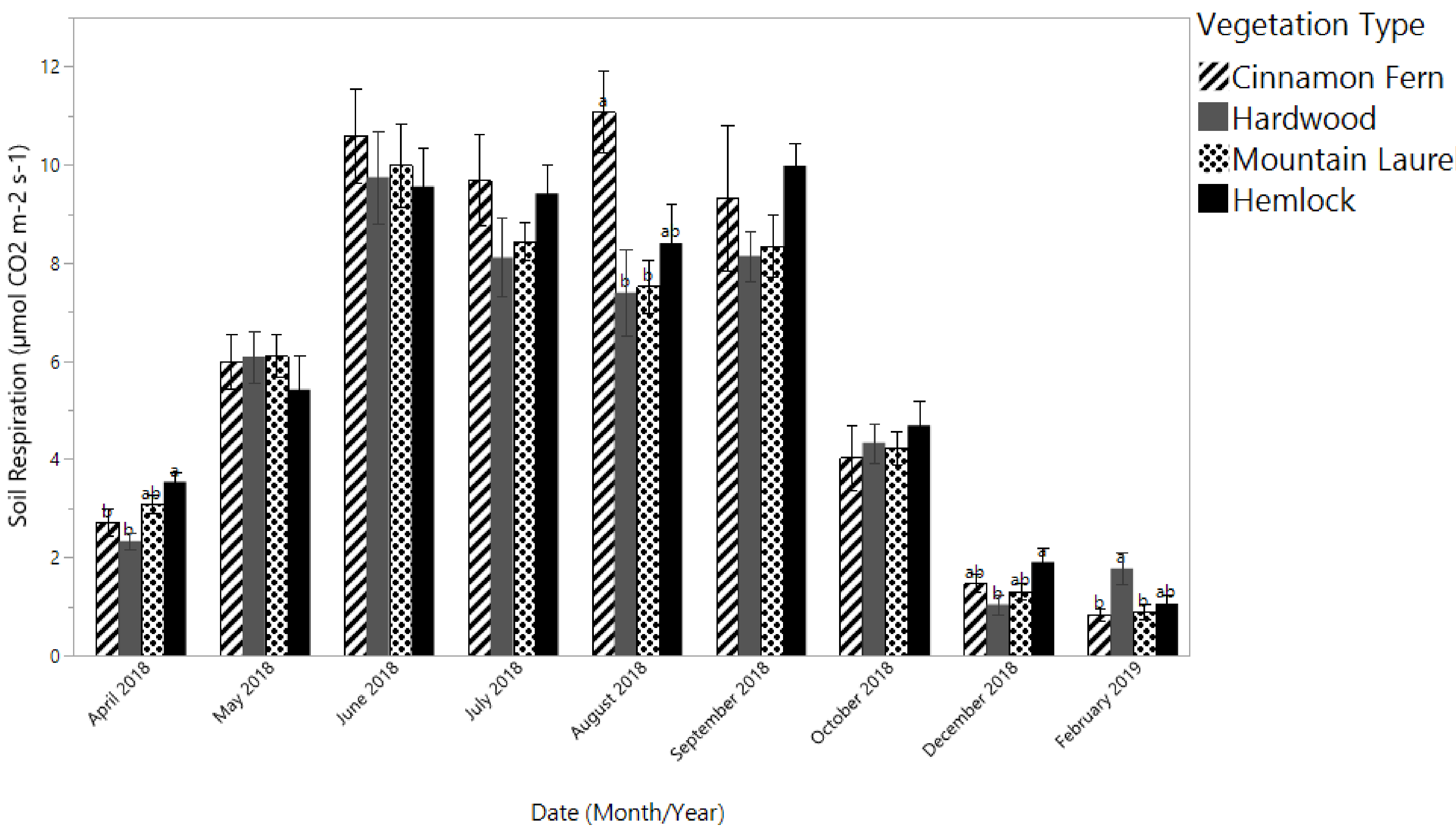

Figure 5. Average soil respiration (R_s) for all sampling dates for hardwood, cinnamon fern, hemlock, and mountain laurel vegetation types. A different letter signifies a significant difference ($P < 0.05$) between vegetation types for that sampling date.

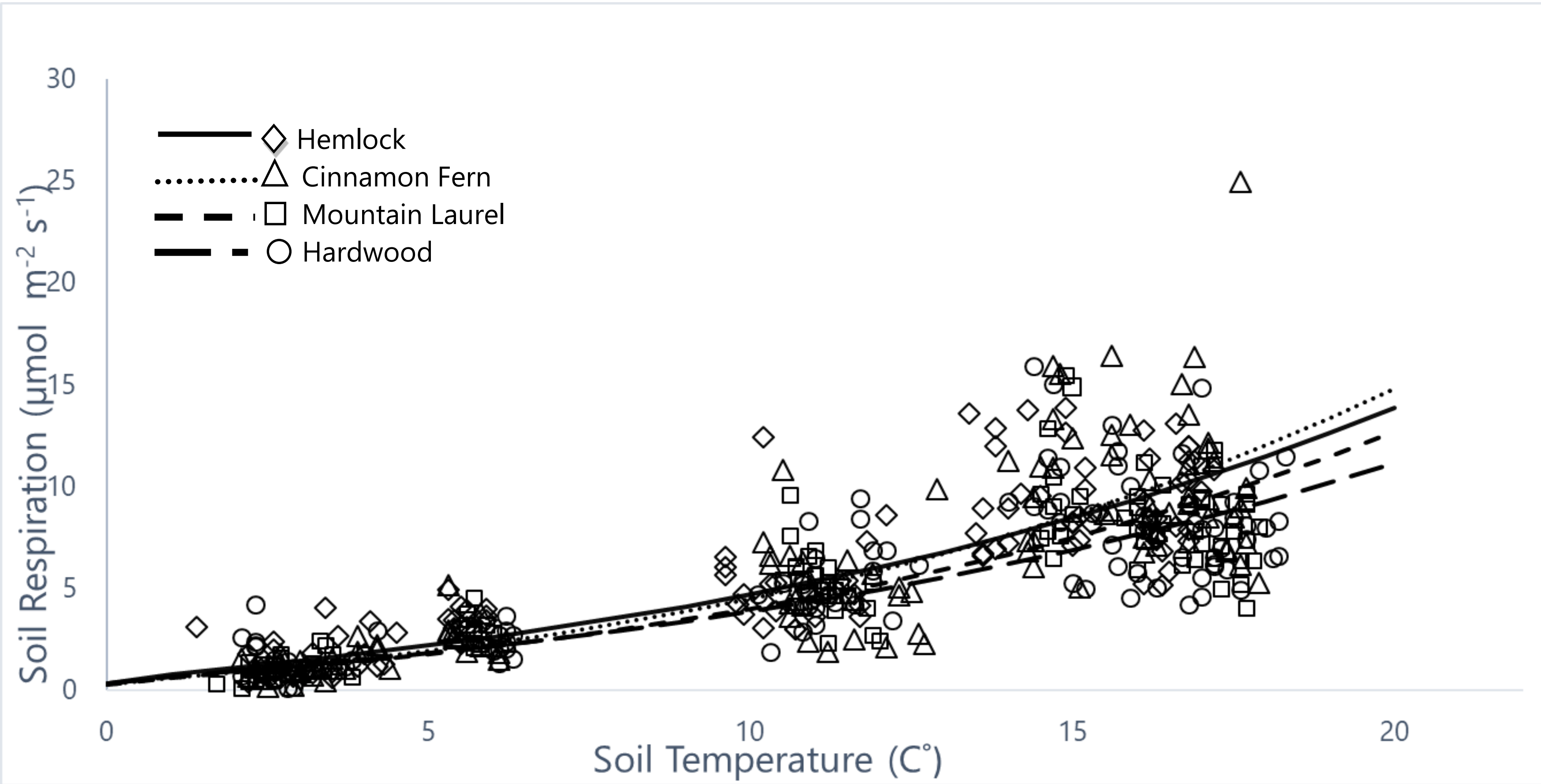

Figure 6. Soil respiration (R_s) in a high elevation southern Appalachian forest (Giles County, VA) as influenced by soil temperature for each vegetation type: hardwood, cinnamon fern, hemlock, and mountain laurel. Predicted lines were generated using the formulas from Table 4 while holding soil moisture at a value of 33.07 (the average of soil moisture for the months of May-September).

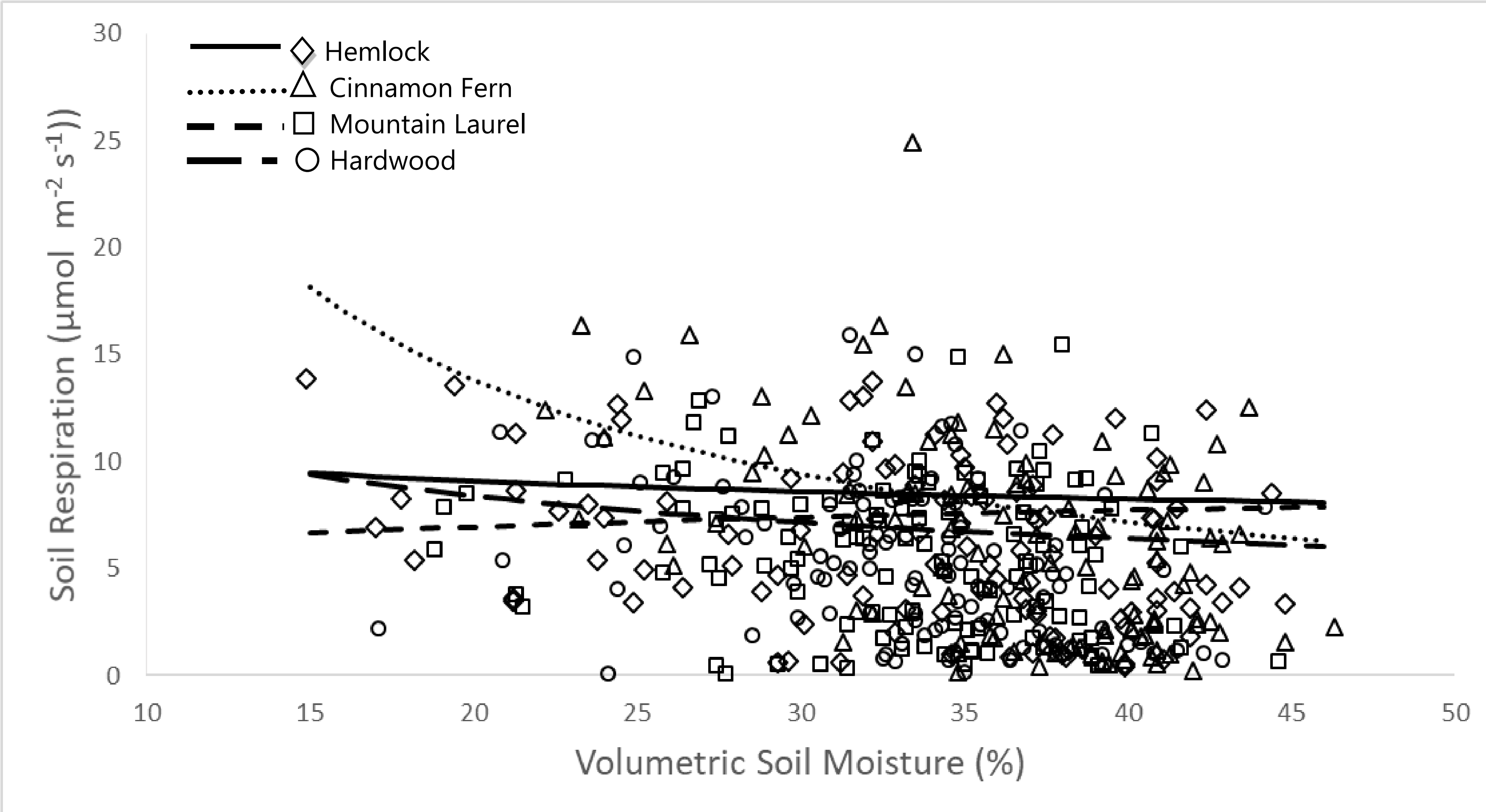

Figure 7. Soil Respiration (R_s) in a high elevation southern Appalachian forest (Giles County, VA) as influenced by soil moisture for each vegetation type: hardwood, cinnamon fern, hemlock, and mountain laurel. Predicted lines were generated using the formulas from Table 4 while holding soil temperature at a value of 15.02 C˚ (the average of soil temperature for the months of May-September).

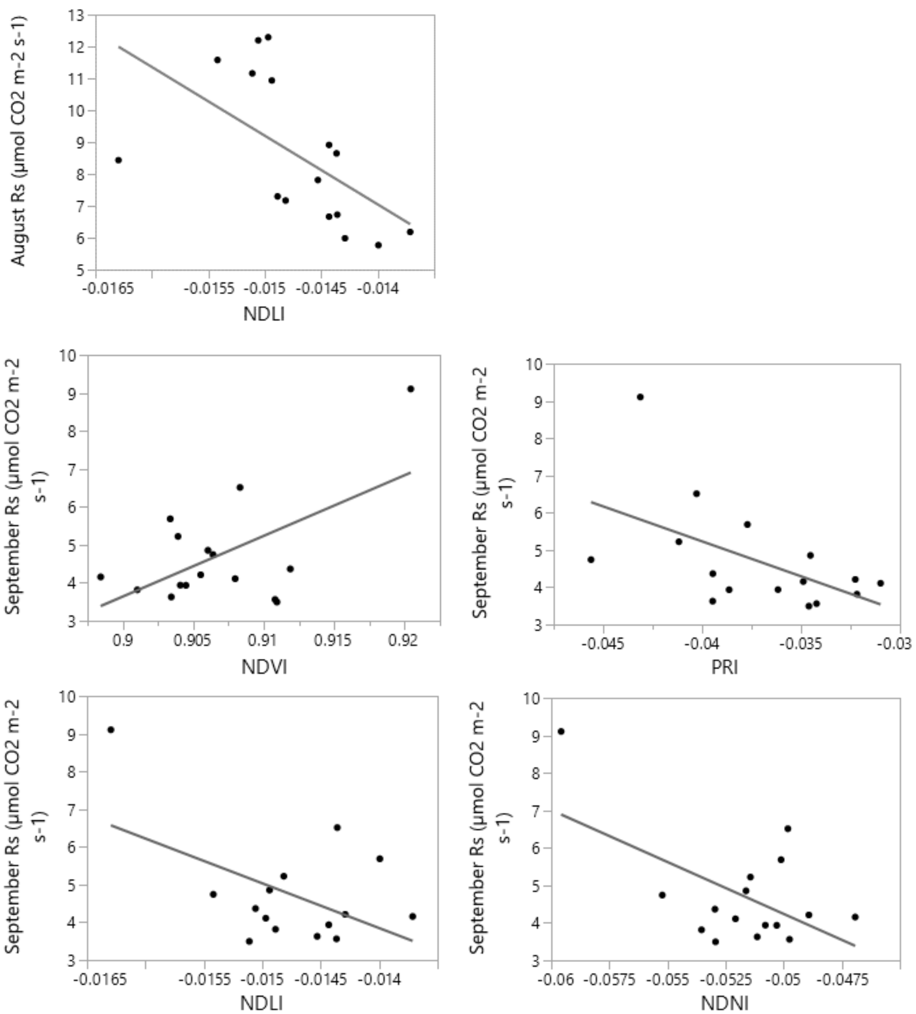

Figure 8. Significant correlations found using Pearson's Correlation between August and September's R_s values for all sixteen plots and NDLI, PRI, NDLI, and NDNI.