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# Variation in foliar nitrogen and albedo in response to nitrogen fertilization and elevated carbon dioxide

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VARIATION IN FOLIAR NITROGEN AND ALBEDO IN RESPONSE  
TO NITROGEN FERTILIZATION AND ELEVATED CO<sub>2</sub>

BY

HALEY F WICKLEIN  
B.A., Earlham College, 2008

Submitted to the University of New Hampshire  
in Partial Fulfillment of  
the Requirements for the Degree of

Master of Science  
in  
Natural Resources

September, 2010

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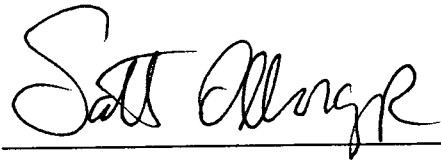
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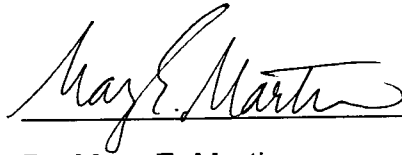
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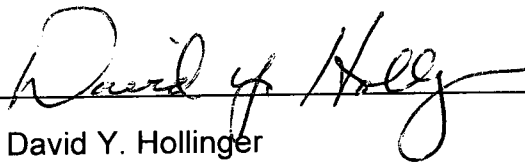
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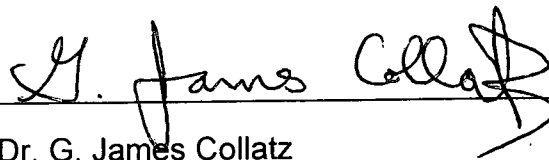
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July 12, 2010

Date

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## **ABSTRACT**

### VARIATION IN FOLIAR NITROGEN AND ALBEDO IN RESPONSE TO NITROGEN FERTILIZATION AND ELEVATED CO<sub>2</sub>

by

Haley F. Wicklein

University of New Hampshire, September, 2010

It has recently been demonstrated that foliar nitrogen is positively correlated with surface albedo over a broad range of plant functional types. However, the mechanism(s) driving the nitrogen- albedo relationship remain elusive. This study investigated leaf spectral properties from three deciduous species subjected to either nitrogen or CO<sub>2</sub> fertilization and compared results to measured chemical and structural properties. We measured reflectance and transmittance, foliar nitrogen, leaf mass per unit area, water content, and  $\delta^{13}\text{C}$  values for stacks of 1, 2, 4, and 8 leaves. Nitrogen was the best predictor of leaf-level albedo of the traits that we measured. There were no significant differences in albedo between CO<sub>2</sub> or nitrogen treatments. Across all species there was a negative relationship between albedo and foliar nitrogen, suggesting that the previously observed nitrogen- albedo relationship is not caused by leaf-level interactions, but is likely due to structural properties at the canopy or stand level.

## CHAPTER I

### INTRODUCTION

Nitrogen (N) is a limiting nutrient for plants (Gruber and Galloway 2008) and a crucial component of the proteins and enzymes needed for all photosynthetic processes (Larcher 1995). As a result, it plays a key role in controlling primary production, leading to highly coupled N and carbon (C) cycles. It has been well documented that leaf-level foliar N and photosynthetic capacity are positively correlated (Field and Mooney 1986, Evans 1989) across a wide range of plant functional types (Reich et al. 1997, Reich et al 1999). Foliar N and photosynthetic capacity also covary at the canopy level across boreal and temperate ecosystems (Ollinger et al. 2008). Recently, Ollinger et al. (2008) and Hollinger et al. (2010) demonstrated that both of these variables are also significantly and positively correlated with growing season shortwave surface albedo (the fraction of incident solar radiation that is reflected by a surface) over a broad range of plant functional types. Bala et al. (2007), with results from a coupled global carbon-cycle and climate deforestation model, suggested that the warming effects of a forest's low albedo can sometimes overwhelm the cooling effects of its potential for C storage. Given the importance of even small changes in surface heat exchange, the occurrence of an N effect on albedo would bear interesting and important consequences for climate.

Although the effects of N deposition on photosynthetic capacity have received considerable attention (e.g., Bauer et al. 2004, Finzi et al. 2006), there are no studies to our knowledge that examine how increased N levels affect albedo and other biophysical factors, and hence the mechanism(s) driving the N-albedo relationship remain elusive. There are many sources of variation in albedo, ranging from chemical and structural properties at the leaf level, to micro-scale structural changes in the canopy (i.e. leaf clumping, leaf inclination angle) to macro-scale differences in the canopy or landscape (i.e. canopy volume, leaf area index, spatial vegetation patterns).

At the leaf level, photosynthetic pigments dominate reflectance in the visible part of the spectrum (350 – 700 nm), water content dominates reflectance in the mid-infrared (MIR, 1350 – 2500 nm), and reflectance in the near infrared (NIR, 700 – 1350 nm) is determined mainly by leaf structure and scattering (Gates et al. 1965). Plants are well adapted to their light environment in that they absorb efficiently in regions where energy is readily usable (i.e. the visible) and poorly where photons lack sufficient energy for photosynthesis (i.e. the NIR), which also serves to reduce their heat load (Gates et al. 1965, Kumar et al. 2001). Consequently, it is the NIR region where the greatest differences in leaf to canopy reflectance patterns are often observed (Sánchez and Canton 1999), and this region is the primary driver of the observed relationship between N and albedo (Ollinger et al. 2008). Therefore, if structural changes within the leaf are driven by, or covary with, foliar N concentration, it is possible that this could be one of the main drivers in the N-albedo relationship observed at the canopy level.

Although it has been observed that N covaries with some leaf structural parameters, clear implications for leaf albedo cannot yet be drawn. For example, Pañuelas et al. (1994) found that foliar N covaried with leaf thickness in sunflowers and Makoto and Koike (2007) found that greater N availability increased leaf mass per unit area (LMA, a proxy for thickness) in Korean pine seedlings. However, Baltzer and Thomas (2005) found that in deciduous saplings, foliar N correlated with palisade mesophyll thickness, but not leaf thickness or LMA. Similarly, Trapáni et al. (1999) found no response of LMA to N fertilization in sunflowers, although they did observe an increase in cell number, cell area, and total leaf area.

Hollinger et al. (2010) hypothesized that the N – albedo relationship is due to increased backscattering (i.e. reflectance) resulting from leaf internal structural changes that are necessary to support rapid CO<sub>2</sub> diffusion and the increased photosynthesis rates made possible by increased foliar N. Particularly important are changes in the ratio of the mesophyll surface area exposed to intercellular air spaces to the area of the leaf ( $A_{mes}/A$ ), which has been shown to be positively correlated with both photosynthetic rates (Nobel et al. 1975, Longstreth et al. 1985) and NIR reflectance (Slaton et al. 2001). Given differences in the refractive indices of hydrated mesophyll cells (refractive index of 1.4 to 1.48) and the intercellular airspace (reflective index of 1.0) (Woolley 1971, Gausman et al. 1974a), a higher  $A_{mes}/A$  value should lead to more opportunities for radiation scattering, and a correspondingly greater chance of the light exiting the leaf. Nobel et al. (1975) showed that the  $A_{mes}/A$  ratio and leaf thickness are positively

correlated, suggesting that measuring leaf thickness may be a way to indirectly assess this leaf trait. Despite these expectations, results in the literature are often contradictory, with some studies measuring higher NIR reflectance for thicker leaves (e.g., Gates et al. 1965, Knapp and Carter 1998, Merzlyak et al. 2004, Castro-Esau et al. 2006) and some observing no significant relationship (e.g., Slaton et al. 2001, Castro and Sanchez-Azofeifa 2008) or even higher NIR reflectance in thinner leaves (e.g., Ceccato et al. 2001).

Long-term N and C fertilization studies provide the opportunity to measure a wide range of N concentration in species growing in similar climatic and environmental conditions. Both leaf- and canopy- level studies (e.g. Reich et al. 1997, Smith et al. 2002, Ollinger et al. 2008) have shown an increase in productivity with increasing foliar N concentration, and low availability of N has been shown to suppress the positive response of plant biomass to elevated CO<sub>2</sub> (Reich 2006). Free air CO<sub>2</sub> enrichment (FACE) sites simulate future concentrations of atmospheric CO<sub>2</sub> by fumigating plots with elevated levels of CO<sub>2</sub>. Data from multiple FACE sites have shown that elevated CO<sub>2</sub> leads to an increase in LMA (Norby et al. 2003) and the consequent dilution of foliar N due to the accumulation of carbohydrates (Oren et al. 2001, Ellsworth et al. 2004, Norby and Iversen 2006). This raises the questions of whether elevated CO<sub>2</sub>, by effecting foliar N levels, could alter a forest's albedo.

Human activities have more than doubled the inputs of N to the terrestrial biosphere (Smil 1990), a trend that is likely to continue or even increase (Gruber and Galloway 2008). Similarly, CO<sub>2</sub> has risen from a preindustrial level of 280

ppm to 379 ppm (IPCC 2007 Synthesis Report) and it is expected to continue to rise well into the future. With the potential for increasing anthropogenic perturbations of the N and C cycles, it is important to understand how changes in N status, through high levels of N and C deposition, could influence a forest's albedo and photosynthetic rates, and therefore its overall feedback (positive or negative) to global warming.

The objective of this study was to examine the degree to which the canopy- level N-albedo relationship stems from leaf-level interactions, and to determine whether leaf albedo is affected by elevated rates of N deposition and CO<sub>2</sub> fertilization. To assess the impact of changes in foliar N concentration on leaf-level optical properties, we measured leaf reflectance and transmittance, as well as leaf chemical and anatomical traits (N concentration, leaf mass per area, equivalent water thickness, water content,  $\delta^{13}\text{C}$ ), for three deciduous species in the eastern United States that have been subjected to either long-term N or CO<sub>2</sub> fertilization. If the albedo-N relationship is driven by changes at the leaf level, we would expect to see higher leaf-level albedo in plots with high N fertilization treatments and lower albedo in plots receiving high CO<sub>2</sub> fertilization than those receiving solely ambient N or CO<sub>2</sub> deposition.

## CHAPTER II

### MATERIALS AND METHODS

#### Study Sites

We measured leaf spectral, chemical, and structural characteristics of leaf samples from two sites in the eastern United States: Harvard Forest, Petersham, MA (42.5°N, 72°W) and Oak Ridge National Laboratory, Roane County, TN (35.9°N, 84.3°W). Harvard Forest contains a long-term N fertilization study. Monthly mean temperatures range from 20 °C in July to -7°C in January. Annual mean precipitation is 110 cm, distributed fairly evenly throughout the year. Ambient nitrogen (N) deposition averages around 8 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Ollinger et al. 1993). The dominant soil types are stony- to sandy-loams formed from glacial till. Elevation is 385 m above sea level.

Oak Ridge National Laboratory in contains both CO<sub>2</sub> and N fertilization plots. Mean annual temperature is around 14°C, and mean annual precipitation is 1371 cm, distributed fairly evenly throughout the year. Ambient N deposition averages between 10 and 15 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Johnson et al. 2004). The dominant soil type is an Aquic Hapludult, a silty clay loam.



## Harvard Forest, MA

Harvard Forest (HF) was established in 1907 and has been a long-term ecological research (LTER) site since 1988. A chronic N amendment experiment was established at HF in 1988, and is maintained by the Forest Ecosystems Study Group at the University of New Hampshire's Complex Systems Research Center. Two adjacent stands were chosen for N additions on the Prospect Hill Tract. The first is a mixed hardwood stand dominated by black oak (*Quercus velutina* Lam.), red oak (*Q. rubra* L.), black birch (*Betula lenta* L.), red maple (*Acer rubrum* L.), and American beech (*Fagus grandifolia* Ehrh.), which regenerated naturally after a clearcut around 1945. The second is an even-aged red pine (*Pinus resinosa* Aiton) stand that was heavily disturbed by an ice storm in December of 2008, and not used in this study.

In each stand, four plots were established: control (no added N), low N (additions of 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>), low N plus sulfur (not included in this study; additions of 50 kg N ha<sup>-1</sup> yr<sup>-1</sup> and 74 kg S ha<sup>-1</sup> yr<sup>-1</sup>), and high N (additions of 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>). Each plot measures 30 x 30 m and is divided into thirty-six 5 x 5 m subplots. Fertilizer additions of ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>) and sodium sulfate (NaSO<sub>4</sub>) began in 1988 as six equal applications over the growing season (May-Sept.). The sulfur additions were terminated in 1998. Starting in 2005, N additions consisted of NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and potassium (K).

## Oak Ridge National Environmental Research Park, TN

The free air CO<sub>2</sub> enrichment (FACE) facility at the Oak Ridge National Laboratory (ORNL) Environmental Research Park is located in a sweetgum (*Liquidambar styraciflua* L.) monoculture that was established in 1988. Dominant understory species include predominantly Japanese stilt grass (*Microstegium vimineum* Trin., a C<sub>4</sub> invasive), with lesser amounts of flowering dogwood (*Cornus florida* L.), Japanese honeysuckle (*Lonicera japonica* Thunb.), autumn olive (*Elaeagnus umbellata* Thunb.) and eastern red cedar (*Juniperus virginiana* L.).

In 1996, a FACE experimental facility was set up in the sweetgum stand. Five 25 m diameter plots were established: two with FACE apparatus emitting elevated CO<sub>2</sub>, two with FACE apparatus but ambient CO<sub>2</sub>, and one with no FACE apparatus. Exposure to elevated CO<sub>2</sub> began in the spring of 1998. Average daytime CO<sub>2</sub> concentration (for 1998 – 2003) is maintained at around 544 ppm for the enriched plots, and averages 391 ppm for the ambient plots (Norby and Iversen 2006).

In 2004, an N fertilization experiment at ORNL was initialized. A 85 m x 50 m area was fertilized in a block pattern within a sweetgum stand planted at the same time as the FACE site sweetgum plantation, but separated by a small stand of sycamore (*Platanus occidentalis* L.). Each block contains two control plots and two fertilized plots (both 12 x 16 m). The fertilized plots receive 200 kg N ha<sup>-1</sup> yr<sup>-1</sup>, applied as urea each year before leaf out (Iversen and Norby 2008).

## **Data Collection and Analysis**

### **Field Sampling: Harvard Forest**

At HF, sampling of foliage from the hardwood plots was conducted at each treatment level between July 20 and July 23, 2009. Within each treatment plot, five red maple and seven black oak individuals were randomly selected and sampled. Using a 12-gauge shotgun, green leaves were collected from the top, middle, and bottom of the canopy. Sample collection heights were determined using a digital hypsometer (Haglöf Vertex). Leaves were placed in plastic Ziploc bags and stored on ice until analysis, which was carried out within 36 hours of collection. In each treatment plot, three litter and two bark samples were collected from each species.

### **Field Sampling: Oak Ridge National Environmental Research Park**

At ORNL, field sampling was conducted between July 28<sup>th</sup> and July 30<sup>th</sup>, 2009. Using a slingshot canopy sampler (N fertilized site) or tower climbing with pole pruners (FACE), we collected green leaves from the top, middle, and bottom of the canopy. Heights were determined using either a digital hypsometer (Haglöf Vertex) or measuring tapes attached to climbers. Within each treatment at the FACE site (ambient and elevated CO<sub>2</sub>) we sampled 10 sweetgum trees. At the N fertilized site, a total of 12 upper canopy and 12 lower canopy samples were collected for each treatment (control and N fertilized). However, due to the physical proximity of the control and N fertilized plots (and consequent N

contamination of the control treatment), we used the adjacent FACE ambient CO<sub>2</sub> plots as our control treatment for statistical analysis. Additionally, understory plants were collected at 1 m intervals on a north-south transect in each treatment, with a visual qualitative assessment to determine percent cover. In all cases, leaves were placed in plastic Ziploc bags and stored on ice until analysis.

### **Spectral data collection**

We measured hemispherical reflectance and transmittance spectra for healthy leaves from each individual using a spectrophotometer (ASD Inc., Boulder, CO) attached to an integrating sphere (SphereOptics), with a halogen bulb light. The ASD spectrophotometer measures reflectance from 350 to 2500 nm, in 1 nm intervals. Measurements were taken for a single leaf and a stack of 2, 4, and 8 leaves (both adaxial and abaxial sides) for each individual tree within several hours of sampling. Reflectance and transmittance spectra of leaf stacks were taken to simulate light movement through canopy layers without the influence of canopy structure. Because of time constraints, we were not able to measure the abaxial reflectance and transmittance for leaf stacks of 2, 4, or 8 for all individuals. Leaves were kept in the same order for all stack measurements. Stem bark and understory reflectance was also measured with the ASD spectroradiometer and integrating sphere. Each reflectance and transmittance sample was determined as the mean of 50 spectral measurements. The spectra were corrected for dark current, and a white reference standard was taken prior

to each set of measurements (reflectance or transmittance of one growing stack of leaves). For reflectance spectra, both the sample and the white reference were kept on the integrating sphere at the same time in order to maintain the integrating sphere's port fraction. However, their port positions were switched between the measurement of the white standard and the sample spectra.

SW spectrally weighted albedo (350 – 2500 nm) values were calculated by multiplying the reflectance or transmittance spectra with the solar spectrum (matching wavelength for wavelength) to create an energy flux spectrum (Fig. 1). The integral of the energy flux spectrum was then divided by the integrated solar spectrum to obtain a value representing the reflected energy as a proportion of incident. For visible (350 – 700 nm), NIR (700 – 1300 nm), and Mid IR (1350 – 2500 nm) reflectance and transmittance values, the same process was followed using only wavelengths from the regions of interest. All reported reflectance and transmittance values are weighted by the solar energy spectrum.

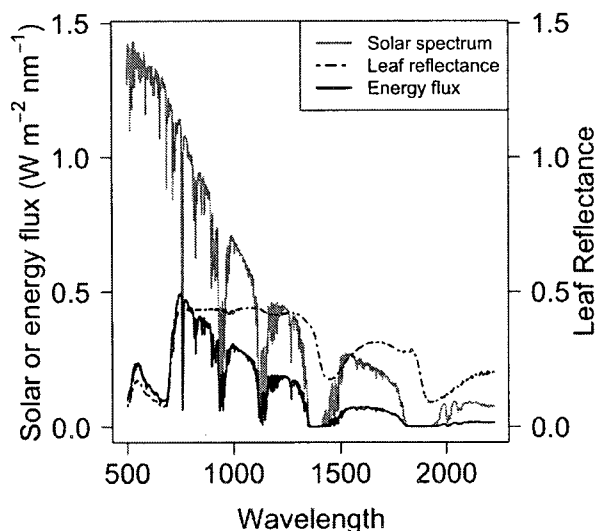


Figure 1. Albedo calculation for a single leaf reflectance spectrum (dashed line, in this case a sweetgum leaf). For each waveband, the reflectance spectrum was multiplied with the solar spectrum (grey line) to create an energy flux spectrum (black line). The integral of the energy flux spectrum was then divided by the integrated solar spectrum to obtain an albedo value representing the reflected energy as a proportion of incident.

## **Leaf Chemical and Structural Analysis**

For measuring water and structural properties, two circular disks (2.035 cm<sup>2</sup> area) were removed from each leaf. These tissue samples were weighed so the amount of leaf tissue for each stack could be calculated (e.g., disks from first leaf, disks from first two leaves, first four leaves, etc.). Leaf disks were then oven dried at 70 °C for at least 72 hrs and weighed again to determine water content (% leaf fresh weight), equivalent water thickness (EWT, measured as g water per cm<sup>2</sup> leaf), and leaf mass per unit area (LMA, measured as g leaf per m<sup>2</sup> leaf).

The remaining leaf sample (after leaf punches were removed) was then ground using a Wiley mill and passed through a 1 mm mesh screen. Prior to N concentration analysis, the ground samples were dried for 24 hours at 70°C. The mass-based leaf-level foliar N concentration ( $N_{\text{mass}}$ , measured as g of N per 100 g of dry leaf matter) was determined from the dried samples using a visible and near infrared spectrophotometer (Foss NIRSystems 6500, Eden Prairie, MN) following the procedure described by Bolster et al. (1996). We determined N per unit leaf area ( $N_{\text{area}}$ ) by multiplying  $N_{\text{m}}$  by the LMA of the sample ( $N_{\text{area}} = N_{\text{mass}} * \text{LMA}$ ).

We also measured leaf  $\delta^{13}\text{C}$  for all samples. Leaf  $^{13}\text{C}$  can become enriched through long – term water stress (Farquhar et al. 1982) or increased photosynthetic rates (Duursma and Marshall 2006). Both of these variables could lead to changes in leaf structure (e.g., Chartzoulakis et al. 2002, Nobel et al. 1975, respectively); therefore  $\delta^{13}\text{C}$  has the potential to be used as an indicator of processes that influence light scattering within the leaf. A positive correlation

between foliar N and  $\delta^{13}\text{C}$  values has been observed in some species (e.g., Livingston et al. 2002, Duursma and Marshall 2006). This relationship could be driven by inherent tradeoffs between nitrogen and water use efficiency (Fredeen et al. 1991) or the importance of foliar N in driving photosynthetic rates (Reich et al. 1997). We measured isotopic  $\delta^{13}\text{C}$  using a Costech Elemental Analyzer coupled to a Thermo Finnigan Delta Plus XP isotope ratio mass spectrometer. Isotope values were expressed as  $\delta^{13}\text{C} (\text{‰}) = ([^{13}\text{C}/^{12}\text{C} \text{ sample}] / [^{13}\text{C}/^{12}\text{C} \text{ standard}] - 1) * 1000\text{‰}$ . Leaves from the elevated  $\text{CO}_2$  treatment at ORNL had extremely low  $\delta^{13}\text{C}$  values (Table 1), however this was likely due to the isotopic signature of the  $\text{CO}_2$  being used for fumigation, not a greater discrimination of  $^{13}\text{CO}_2$ . Therefore,  $\delta^{13}\text{C}$  data from the elevated  $\text{CO}_2$  treatment was excluded in subsequent analysis.

### **Statistical Analysis**

Summary statistics (means and standard error) were computed for all optical properties and leaf traits. The significances of the mean differences between treatments were determined by analysis of variance (ANOVA), with pairwise comparisons tested using Tukey's 'Honest Significant Difference' method. Regression analysis was used to determine relationships between optical properties and leaf traits. For multiple regressions the adjusted  $r^2$  was considered instead of  $r^2$  because this statistic penalizes the model for an increased number of parameters, thereby decreasing the likelihood of overfitting.

Prior to analysis, variables were tested for normality with the Shapiro-Wilk statistic and in subsequent analysis, variables were power transformed where needed to correct for skew. Single leaf SW and NIR albedo were non-normal due to a small number of outliers (determined by  $1.5 \times$  interquartile range). Statistical tests were performed with and without the outliers, and because the resulting explained variances were similar, outliers were removed to follow assumptions of normality in linear regression models. All statistical analysis was completed using the software R, version 2.8.1 (R Foundation for Statistical Computing 2008). Results were considered statistically significant at the 0.05 level. Reported results are for single leaves, adaxial side, unless otherwise specified.



## CHAPTER III

### RESULTS

#### Adaxial vs. Abaxial Leaf Side

Similar to results from other studies (e.g. Woolley 1971) the adaxial side reflected less in the visible than the abaxial side, but for transmittance the pattern was reversed ( $p < 0.001$  in both cases). There were no significant differences between sides for either NIR reflectance or transmittance ( $p > 0.1$ ). The mid IR did not differ significantly between sides for reflectance, but transmittance was slightly higher for the abaxial side. This pattern was consistent for all species, treatments, and canopy heights. A two-way ANOVA showed no side –  $N_{\text{mass}}$  interaction for any of the species; although there are differences in reflectance between leaf sides, this does not change based on the N content of the leaf. Given an absence of an N - leaf side interaction, the remainder of the paper is constrained to measurements from the adaxial leaf side.

## Treatment Differences

### Leaf Traits

#### Harvard Forest

For red maple and black oak,  $N_{\text{mass}}$  was higher in the high N treatment than the low N and control treatments ( $p < 0.001$ , Fig. 2d-e, Table 1). There were no differences in LMA between treatments for black oak or red maple individuals ( $p > 0.1$ , Fig. 2g-h, Table 1). EWT did not differ between treatments for any species ( $p > 0.1$ , Table 1).  $\delta^{13}\text{C}$  was only significantly different between treatments for black oaks, with the control treatment being more depleted in  $^{13}\text{C}$  than the high N treatment ( $p < 0.05$ , Table 1).

#### Oak Ridge National Laboratory

Similar to results from HF,  $N_{\text{mass}}$  was higher in the N fertilized treatment than the ambient or elevated  $\text{CO}_2$  treatments (which were not significantly different from each other) ( $p < 0.001$ , Fig. 2f, Table 1). The N fertilized treatment at ORNL had significantly lower LMA than the ambient or elevated  $\text{CO}_2$  treatments ( $p > 0.1$ , Fig. 2i, Table 1). EWT and  $\delta^{13}\text{C}$  did not differ between treatments for any species ( $p > 0.1$  in both cases, Table 1).

## Optical Properties

### Harvard Forest

There were no differences between treatments in SW reflectance (Fig. 2a-b) or transmittance, NIR reflectance or transmittance, Mid IR reflectance and transmittance, or SW absorption (1-[reflectance + transmittance]) for any species ( $p > 0.1$  in all cases). Visible reflectance and transmittance followed differences in N between treatments, with lower values corresponding to higher N fertilization ( $p < 0.05$  in all cases).

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There were no differences between treatments in SW reflectance (except for higher mean values in the ORNL elevated treatment than ORNL N fertilized treatment, Fig. 2c), NIR reflectance, SW transmittance, or SW absorption (1-[reflectance + transmittance]) for any species ( $p > 0.1$  in all cases). NIR transmittance in the ORNL N fertilized treatment was higher than in the ambient or elevated CO<sub>2</sub> treatments ( $p < 0.01$ ). Visible reflectance and transmittance followed differences in N between treatments, with lower values corresponding to higher N fertilization ( $p < 0.05$  in all cases). Mid IR reflectance and transmittance were both higher for N fertilized sweetgums ( $p < 0.01$ ), likely due to differences in LMA and EWT.

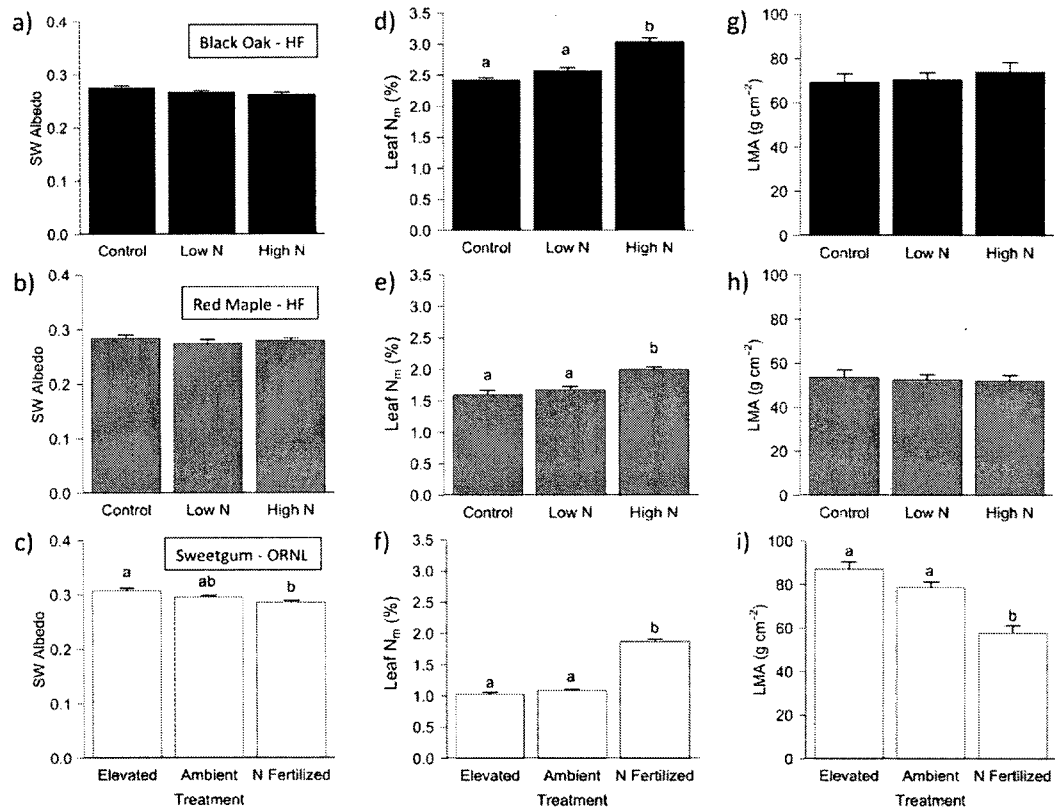


Figure 2. ANOVA results (means  $\pm$  standard error) for SW Albedo (a-c),  $N_{mass}$  (d-f), and LMA (g-h) treatment differences. Results for black oak (site: HF) are depicted with black bars, red maple (site: HF) in grey bars, and sweetgum (site: ORNL) in white bars. Means with different letters were significantly different in pair-wise comparisons (Tukey's multiple comparison test).

Table 1. Means ( $\pm$  standard error) of measured leaf traits within each treatment for all three species studied (black oak = BO, red maple = RM, and sweetgum = SG).

Species	Treatment	N <sub>mass</sub> (%)	N <sub>area</sub> (g m <sup>-2</sup> )	LMA (g m <sup>-2</sup> )	EWT (mg cm <sup>-2</sup> )	$\delta^{13}\text{C}$
BO	High N	3.04 $\pm$ 0.06	2.25 $\pm$ 0.14	74.0 $\pm$ 4.3	8.97 $\pm$ 0.52	-28.1 $\pm$ 0.26
	Low N	2.58 $\pm$ 0.04	1.82 $\pm$ 0.09	70.6 $\pm$ 3.0	9.80 $\pm$ 0.36	-28.8 $\pm$ 0.20
	Control	2.42 $\pm$ 0.03	1.69 $\pm$ 0.10	69.4 $\pm$ 2.5	9.41 $\pm$ 0.49	-28.9 $\pm$ 0.24
RM	High N	2.00 $\pm$ 0.04	1.04 $\pm$ 0.05	52.1 $\pm$ 2.5	7.22 $\pm$ 0.28	-29.6 $\pm$ 0.25
	Low N	1.68 $\pm$ 0.05	0.90 $\pm$ 0.05	52.6 $\pm$ 2.4	6.92 $\pm$ 0.20	-29.8 $\pm$ 0.13
	Control	1.60 $\pm$ 0.06	0.86 $\pm$ 0.07	53.7 $\pm$ 3.5	6.78 $\pm$ 0.38	-29.9 $\pm$ 0.27
SG	Elevated CO <sub>2</sub>	1.02 $\pm$ 0.04	0.86 $\pm$ 0.04	87.1 $\pm$ 3.3	11.84 $\pm$ 0.33	-41.4 $\pm$ 0.43
	Ambient CO <sub>2</sub>	1.09 $\pm$ 0.02	0.86 $\pm$ 0.04	78.7 $\pm$ 2.6	11.94 $\pm$ 0.25	-31.0 $\pm$ 0.16
	N Fertilized	1.87 $\pm$ 0.04	1.07 $\pm$ 0.06	57.7 $\pm$ 3.5	11.68 $\pm$ 0.39	-31.0 $\pm$ 0.28

## Relationships Between N and Optical Properties

The relationships between both  $N_{\text{mass}}$  and  $N_{\text{area}}$  and reflectance and transmittance of all optical regions were qualitatively similar across sites and treatments, however the explained variances were lower for  $N_{\text{area}}$  (Table 2). An increase in  $N_{\text{area}}$  could be due to either an increase in LMA or and increase in  $N_{\text{mass}}$ , however  $N_{\text{mass}}$  and LMA generally exhibit opposite trends (i.e. LMA is positively correlated with visible reflectance, while the relationship between  $N_{\text{mass}}$  and visible reflectance is negative). Therefore it is probable that the similarities between the  $N_{\text{mass}}$  and  $N_{\text{area}}$  relationships are in fact driven by changes in  $N_{\text{mass}}$ , but mediated by the opposing affects of LMA. Because the changes in  $N_{\text{area}}$  can be explained by these two variables, the subsequent analysis and discussion of foliar N and optical properties will focus on  $N_{\text{mass}}$ .

Table 2. Comparison of regression statistics for foliar N on a mass ( $N_{\text{mass}}$ , %) and area basis ( $N_{\text{area}}$ ,  $\text{g m}^{-2}$ ). This table reports the coefficient of determination ( $r^2$ ), p value, and the sign of the slope of the regression line ('Sign' column) for each model. Insignificant trends are represented by 'ns.' All species and sites were included in the regression analyses. Both  $N_{\text{mass}}$  and  $N_{\text{area}}$  exhibit similar trends, although the explained variances are lower for  $N_{\text{area}}$ , likely due to the opposing effects of LMA.

Response Variable	Source of Variation					
	$N_{\text{mass}}$			$N_{\text{area}}$		
	$r^2$	p value	Sign	$r^2$	p value	Sign
SW Albedo	0.29	< 0.001	-	0.17	< 0.001	-
SW Transmittance	0.1	< 0.001	+		ns	
NIR Reflectance	0.17	< 0.001	-	0.08	< 0.001	-
NIR Transmittance	0.25	< 0.001	+	0.08	< 0.001	+
Mid IR Reflectance		ns		0.06	< 0.001	-
Mid IR Transmittance	0.32	< 0.001	+		ns	
VIS Reflectance	0.54	< 0.001	-	0.18	< 0.001	-
VIS Transmittance	0.28	< 0.001	-	0.25	< 0.001	-

Furthermore, although we did sample leaves from three different heights in the canopy,  $N_{\text{mass}}$  was not correlated with canopy height within species or treatments for BO or RM ( $p > 0.1$  in all cases).  $N_{\text{mass}}$  was negatively correlated with canopy height in the elevated SG treatment ( $r^2 = 0.14$ ,  $p < 0.05$ ), however there was no relationship between  $N_{\text{mass}}$  and canopy height in either the nitrogen fertilized or ambient  $\text{CO}_2$  treatments ( $p > 0.05$  in both cases). Therefore, it is likely that the height from which a leaf was collected is not driving the wide range in foliar N observed within each species, and so the following relationships include leaf measurements from all canopy heights.

Across all species,  $N_{\text{mass}}$  was the single best single predictor of SW, NIR, and visible reflectance and transmittance for all species combined (Fig. 3 a-c). However, contrary to our expectations, for all the spectral regions above, the relationship between  $N_{\text{mass}}$  and reflectance were negative (i.e. the more N present in the foliage, the lower the reflectance). There was a positive relationship between both SW and NIR transmittance and  $N_{\text{mass}}$ , whereas the relationship between  $N_{\text{mass}}$  and visible transmittance was negative (Fig. 3 d-f). Although  $N_{\text{mass}}$  was not the best predictor for Mid IR reflectance or transmittance (EWT was, see below), the relationship was significant and positive for Mid IR transmittance ( $r^2 = 0.32$ ,  $p < 0.001$ ). There was no significant relationship between  $N_{\text{mass}}$  and Mid IR reflectance ( $p > 0.1$ ). Absorption was negatively correlated with  $N_{\text{mass}}$  in the visible ( $r^2 = 0.48$ ,  $p < 0.001$ ), positively in the Mid IR ( $r^2 = 0.24$ ,  $p < 0.001$ ), and very weakly positively in the NIR ( $r^2 = 0.04$ ,  $p < 0.01$ ), leading to no significant relationship between total SW absorption and  $N_{\text{mass}}$ .

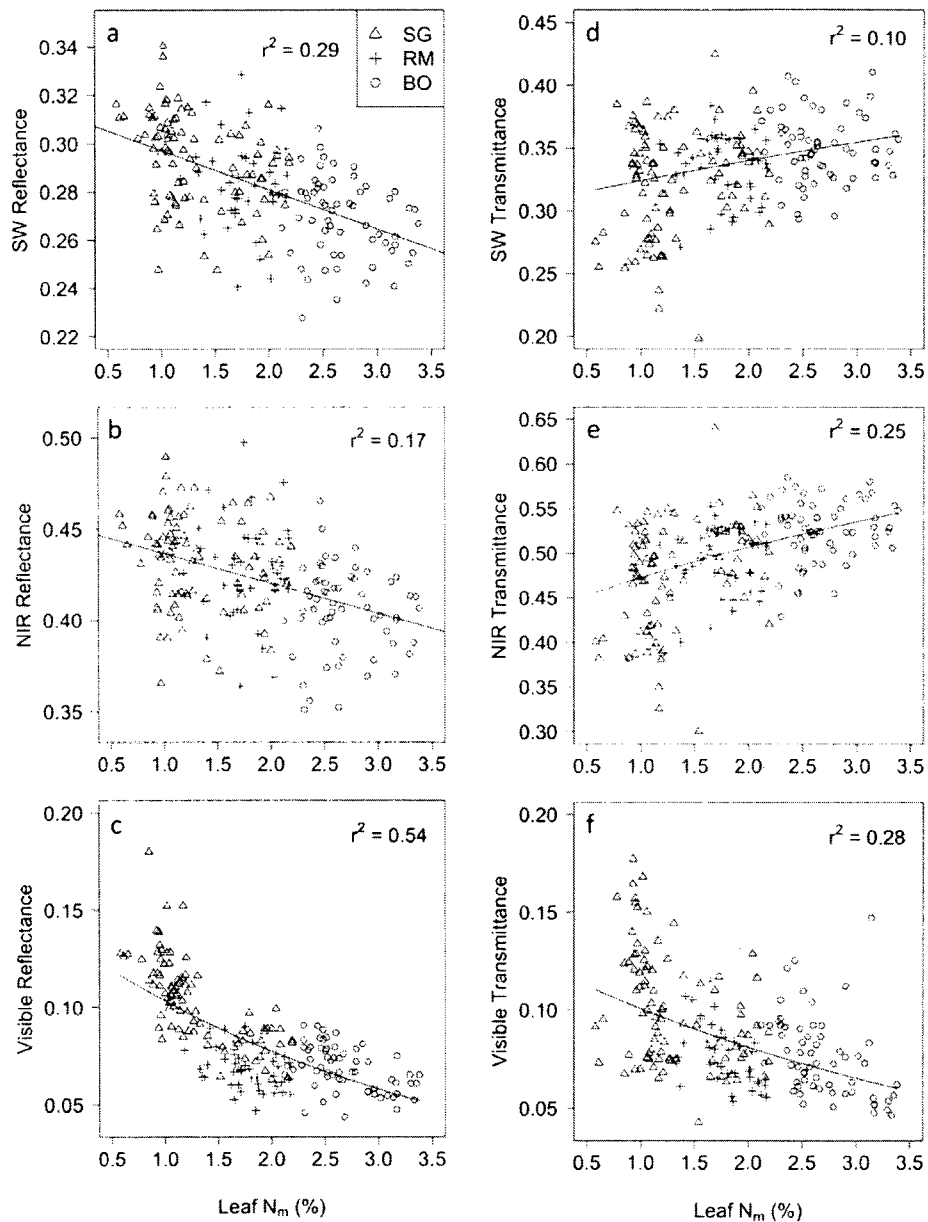


Figure 3. Correlations between foliar  $N_{mass}$  and reflectance and transmittance albedo for (a) SW (350-2500 nm), (b) NIR (700-1300 nm), and (c) visible (350-700 nm) reflectance, and (d) SW, (e) NIR, and (f) visible transmittance. All correlations are significant at  $p < 0.001$ . Each plot includes data from all three species: sweetgum ( $\Delta$ ), red maple (+), and black oak (o).



The above results describe how  $N_{\text{mass}}$  varied with reflectance and transmittance for a single leaf. As the number of leaves in each stack increased, the correlation coefficients for the relationships between  $N_{\text{mass}}$  and all optical properties decreased, however, the overall patterns (i.e. sign of the slope of the regression line) remained the same for all leaf stacks (Fig. 4).

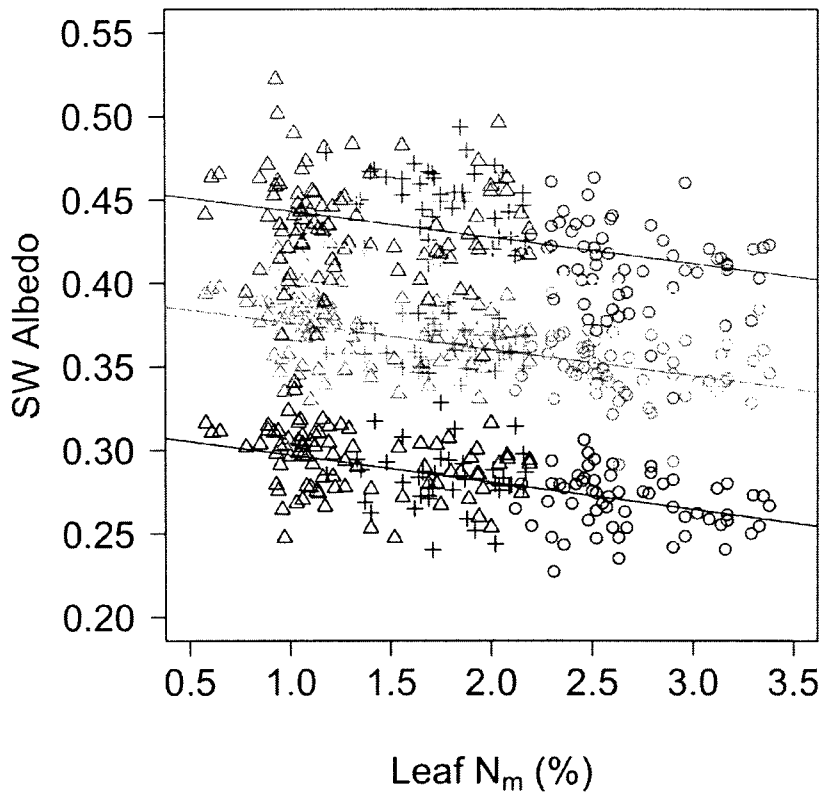


Figure 4. Foliar  $N_{\text{mass}}$  versus SW reflectance albedo for stacks of one (black,  $r^2 = 0.29$ ,  $p < 0.001$ ), two (red,  $r^2 = 0.26$ ,  $p < 0.001$ ), four (green,  $r^2 = 0.18$ ,  $p < 0.001$ ), and eight (blue,  $r^2 = 0.13$ ,  $p < 0.001$ ) leaves. Black oaks (o), red maples (+), and sweetgums ( $\Delta$ ) were all included in the regression analysis. Although the y-intercept changes, all stacks show similar negative relationships between foliar N and SW reflectance albedo.

### **Relationships Between Leaf Traits and Optical Properties**

There was a weak, but significant, positive relationship between LMA and SW reflectance, and weak negative relationships between LMA and both SW and NIR transmittance (Fig. 5). There were stronger negative correlations between LMA and Mid IR reflectance ( $r^2 = 0.38$ ,  $p < 0.001$ ) and transmittance ( $r^2 = 0.40$ ,  $p < 0.001$ ), although this is likely due to the influence of EWT (see below). Visible reflectance was positively correlated with LMA ( $r^2 = 0.20$ ,  $p < 0.001$ ), whereas there was no relationship between visible transmittance and LMA ( $p > 0.1$ ).

Canopy height (the height in the canopy from which each leaf was collected) was positively correlated with visible reflectance ( $r^2 = 0.12$ ,  $p < 0.001$ ) and negatively correlated with both Mid IR reflectance and transmittance ( $r^2 = 0.18$  and  $0.23$  and  $p < 0.001$ , respectively). These relationships are most likely due to the response of the optical properties to changes in LMA, which was positively correlated with canopy height ( $r^2 = 0.61$  across all species).

EWT was the single best predictor for Mid IR reflectance and transmittance (Fig. 6). Although the coefficients of determination were weaker, EWT was also positively correlated with SW ( $r^2 = 0.06$ ,  $p < 0.01$ ), NIR ( $r^2 = 0.04$ ,  $p < 0.01$ ), and visible reflectance ( $r^2 = 0.28$ ,  $p < 0.001$ ), and negatively correlated with SW ( $r^2 = 0.10$ ,  $p < 0.001$ ) and NIR transmittance ( $r^2 = 0.09$ ,  $p < 0.001$ ).

Relative water content (%) was not correlated with any optical parameter that we considered ( $p > 0.1$ ). Other studies (e.g., Datt 1999, Ceccato et al. 2001) have also demonstrated that EWT is strongly related to IR reflectance for many

vegetation types, whereas the correlation between water content and IR reflectance is often weak to nonexistent.

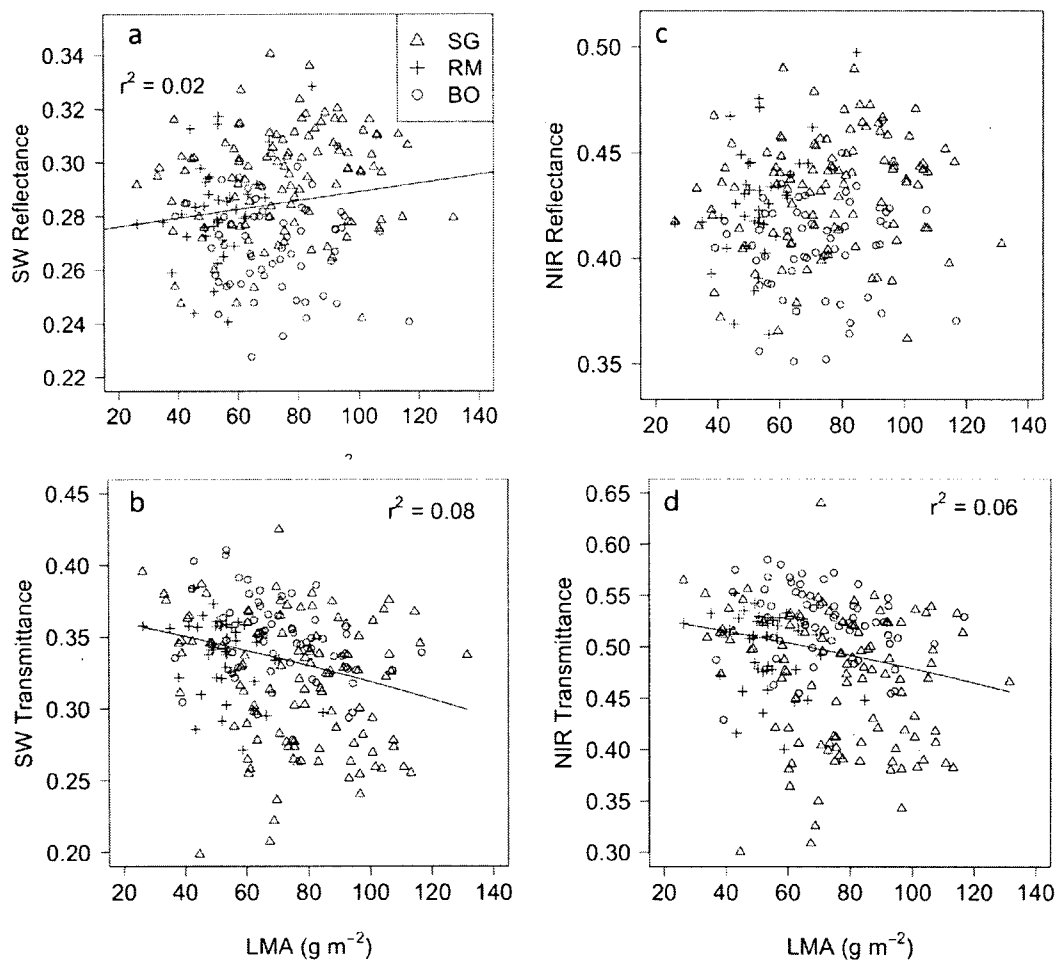


Figure 5. Correlations between LMA and (a) SW reflectance ( $p < 0.05$ ), (b) SW transmittance ( $p < 0.001$ ), (c) NIR reflectance (not significant), and (d) NIR transmittance ( $p < 0.001$ ). Relationship between LMA and NIR reflectance is not significant. LMA explains little to no variation in NIR or SW albedo. Black oaks (o), red maples (+), and sweetgums ( $\Delta$ ) were all included in the regression analysis.

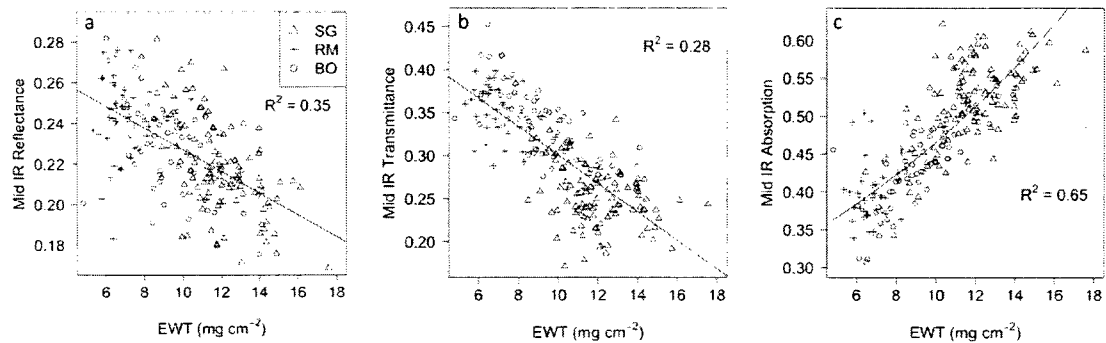


Figure 6. Both reflectance (a) and transmittance (b) declined in the Mid IR region as leaf EWT increased. This was due to the strong positive correlation between Mid IR absorption ( $1 - [\text{reflectance} + \text{transmittance}]$ ) and EWT (c), which demonstrates the importance of water absorption in the Mid IR in influencing scattering from this region. All correlations are significant at  $p < 0.001$ . Black oaks (o), red maples (+), and sweetgums ( $\Delta$ ) were all included in the regression analysis.

$\delta^{13}\text{C}$  was positively correlated with height in all species ( $r^2 = 0.06, 0.27,$  and  $0.55$  and  $p < 0.1, 0.001,$  and  $0.001$  for black oak, red maple, and sweetgum respectively). Overall,  $\delta^{13}\text{C}$  was negatively correlated with SW reflectance ( $r^2 = 0.12, p < 0.001$ ). The relationship between  $\delta^{13}\text{C}$  and SW reflectance was likely driven the stronger negative correlation between  $\delta^{13}\text{C}$  and visible reflectance ( $r^2 = 0.23, p < 0.001$ ), although  $\delta^{13}\text{C}$  was very weakly, but negatively correlated with NIR ( $r^2 = 0.04, p < 0.05$ ) and Mid IR reflectance as well ( $r^2 = 0.07, p < 0.001$ ).  $\delta^{13}\text{C}$  was only significantly correlated with transmittance in the visible region ( $r^2 = 0.31, p < 0.001$ ). Positive correlations between  $\delta^{13}\text{C}$  and both  $N_{\text{mass}}$  and  $N_{\text{area}}$  were also observed across all sites and species (Fig. 7).

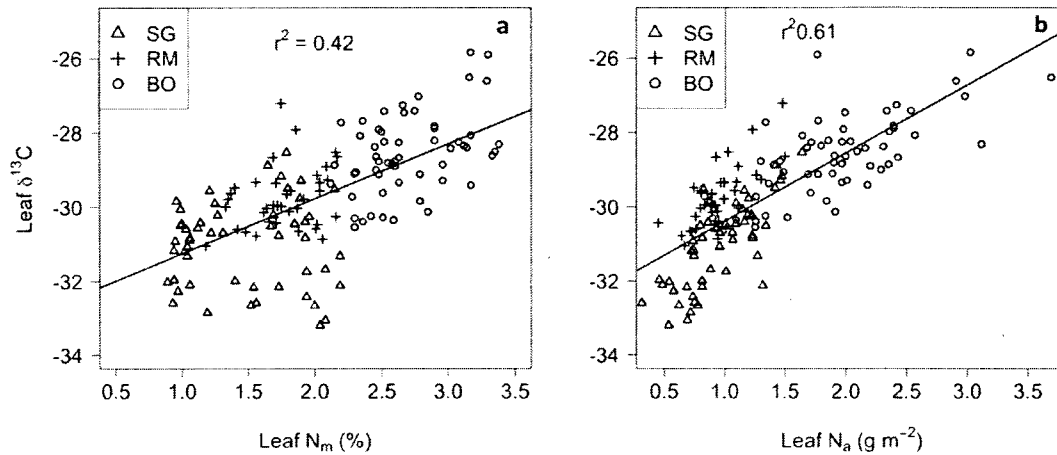


Figure 7. Positive correlation between  $\delta^{13}\text{C}$  and both (a)  $N_{\text{mass}}$  and (b)  $N_{\text{area}}$  across sites and species. Both relationships are significant at  $p < 0.001$ . Black oaks (o), red maples (+), and sweetgums ( $\Delta$ ) were all included in the regression analysis.

Although there were significant correlations between SW reflectance and the leaf traits other than  $N_{\text{mass}}$ , the measured leaf traits explained none of the scatter from the regression between  $N_{\text{mass}}$  and SW reflectance. We compared the residuals of the  $N_{\text{mass}}$  – SW reflectance correlation against each of the other measured leaf traits, none of which were significantly correlated with the residuals ( $p > 0.05$  in all cases), and none explained more than 1% of the variation. The same pattern was found when comparing the leaf traits against the residuals of the  $N_{\text{mass}}$  – NIR reflectance model. Similar results were obtained when the leaf traits were compared to the SW and NIR transmittance –  $N_{\text{mass}}$  models, however the explanatory power was slightly higher (2-7% for canopy height, EWT, and  $^{13}\text{C}$ ).

SW reflectance predictions were only slightly improved through multiple regression models. Models that included  $N_{\text{mass}}$  and either LMA, EWT, canopy height, or some combination of these three parameters improved the prediction, but only slightly ( $r^2 = 0.31$  as compared to an  $r^2$  of 0.29 for a linear model between SW albedo and  $N_{\text{mass}}$ ). The best SW reflectance prediction was obtained with a model including  $N_{\text{mass}}$ , LMA, and EWT ( $r^2 = 0.31$ ,  $p < 0.001$ ). However, the interaction term between LMA and EWT was marginally significant ( $p < 0.1$ ) suggesting that the effects of these two parameters may not be completely independent. No other combinations of leaf traits increased the explanatory power of the prediction model.

The ratio between NIR reflectance and NIR transmittance (the scattering coefficient, Allen et al. 1970) gives insight into the number of refractive interfaces between cell walls and the intercellular airspace. As the amount of intercellular airspace (and hence the number of refractive surfaces) increases, the probability that light will be reflected increases while the probability that it will be transmitted simultaneously decreases, causing the scattering coefficient to increase. The scattering coefficient was negatively correlated with  $N_{\text{mass}}$  (Fig. 8). It was also slightly positively correlated with LMA, EWT, and height, and slightly negatively with  $\delta^{13}\text{C}$ , however none of these variables explained more than 10% of the variance.

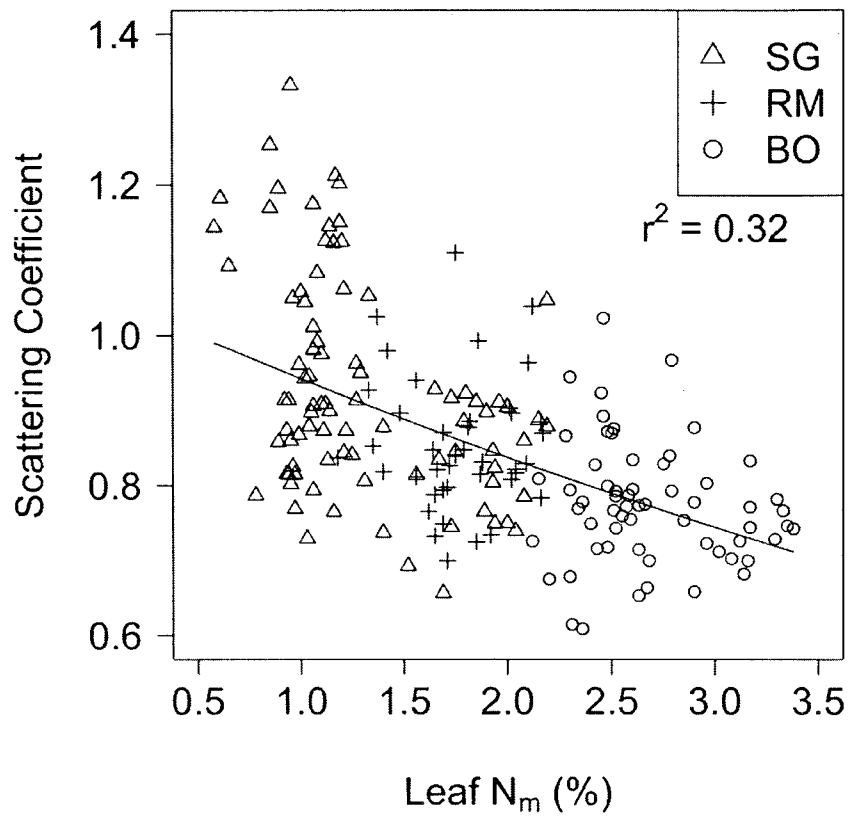


Figure 8. Negative correlation between  $N_{mass}$  and the scattering coefficient (NIR reflectance / NIR transmittance) ( $p < 0.001$ ). The scattering coefficient gives insight into the number of refractive interfaces between cell walls and the intercellular airspace within a leaf.

## CHAPTER IV

### DISCUSSION AND CONCLUSIONS

The goal of this study was to examine leaf-level relationships that might help to explain a previously observed positive relationship between canopy N and shortwave surface albedo (Ollinger et al. 2008, Hollinger et al. 2010). Our initial hypothesis, that we would observe differences in leaf albedo between fertilization treatments, was not substantiated by the results of this study. There were no differences in leaf SW reflectance or transmittance between comparable treatments for both the N and CO<sub>2</sub> fertilization plots. Although there were no differences in leaf albedo between CO<sub>2</sub> fertilization treatments, we also observed no differences in mean foliar N between the ambient and elevated CO<sub>2</sub> fertilization plots, and hence our results neither support nor refute our hypothesis that leaf albedo would decrease with increased CO<sub>2</sub> fertilization. Differences in foliar N between CO<sub>2</sub> treatments have been observed in the past at ORNL (Norby and Iversen 2006); however, after 11 years of CO<sub>2</sub> fertilization, differences between treatments appear to be diminishing. Nevertheless, the absence of differences in albedo between the N fertilization treatments at HF, despite observed differences in foliar N<sub>mass</sub>, indicate that we are correct in rejecting our initial hypothesis.



However, although no treatment differences were observed for leaf albedo,  $N_{\text{mass}}$  was the best predictor of leaf-level reflectance and transmittance of the leaf traits that we measured. With all species combined, there was a significant negative relationship between foliar  $N_{\text{mass}}$  and reflectance, and a positive relationship between foliar  $N_{\text{mass}}$  and transmittance. Hollinger (unpublished) also observed a similar negative relationship between foliar N and both SW and NIR reflectance at the leaf level across 20 deciduous tree species (Table 3). Leaf SW radiation scattering is caused by a combination of pigment concentration and leaf structural properties (Buschmann et al. 1990). Therefore, the negative relationship between foliar  $N_{\text{mass}}$  and leaf-level reflectance is likely driven in part by the visible spectrum, where high N foliage absorbs more light due to increased pigment concentration and hence has lower reflectance. Incoming solar radiation contains more energy in the visible region than other regions of the SW spectrum (Fig.1). Therefore, visible reflectance has a greater influence on SW reflectance than might be expected given the generally low reflectance values recorded from vegetation in this region. Nevertheless, when restricted solely to the NIR region, there was still a negative, although slightly weaker, relationship between leaf-level reflectance and foliar  $N_{\text{mass}}$ . This runs contrary to our prediction that leaves with higher foliar N would have higher leaf reflectance, particularly in the NIR region.

NIR scattering is thought to be due to leaf structural parameters such as leaf thickness, the amount of intercellular air space (%IAS), or the  $A_{\text{mes}}/A$  ratio (Knapp and Carter 1998, Gausman et al. 1970, Slaton et al. 2001). Our study

Table 3. Comparison of regression statistics between this study and data from Hollinger (unpublished), which included 20 different deciduous tree species. Both studies measured single leaf reflectance using a spectrophotometer attached to an integrating sphere. Data from both studies show a negative relationship between both SW and NIR leaf albedo and foliar  $N_{\text{mass}}$ .

Study	Optical Region	$r^2$	p value	Slope of regression line
Wicklein	SW	0.29	< 0.001	-0.019
	NIR	0.17	< 0.001	-0.021
Hollinger	SW	0.20	< 0.001	-0.014
	NIR	0.15	< 0.001	-0.019

found NIR reflectance was correlated with LMA (often used as a proxy for thickness), although the relationship was weak ( $R^2 < 0.05$ ). However, LMA is a product of both the thickness and the density of leaves, and the two parameters have opposing effects: increased leaf thickness increases %IAS, whereas increased leaf density decreases %IAS (Niinemets 1999, 2001). Castro-Díez et al. (1997) found LMA to correlate better with density in some species and thickness in others, and Knapp and Carter (1998) found that LMA has less explanatory power than thickness when considering variation in NIR light scattering. It is possible that the confounding effects of density and thickness in the LMA measurements prevented us from obtaining a clear understanding of the structural parameters affecting leaf radiation scattering. For example, although black oaks (the species with the highest foliar N) had a high mean LMA (Table 1), this could actually be due to increased leaf density, leading to less potential for internal light scattering. Castro-Esau et al. (2006) found that species with

leaves that had low %IAS also had lower reflectance and higher transmittance in the NIR region compared to leaves with high %IAS. The same pattern was also seen as the %IAS developed throughout the maturation of cotton leaves (Gausman et al. 1970). It is possible that this is also the mechanism driving the observed patterns in leaf reflectance and transmittance for the species we sampled.

Our results suggest that, at least to some extent, foliar N directly affects, or covaries with, structural differences in leaves in such a way that leaf reflectance is reduced with increasing foliar N. For example, a strong positive correlation between foliar N and  $\delta^{13}\text{C}$  has been observed in many conifer species (Duursma and Marshall 2006) and nitrogen stress has been shown to decrease water use efficiency and  $\delta^{13}\text{C}$  in white spruce (Livingston et al. 2002). Similarly, a positive correlation between  $\delta^{13}\text{C}$  and N on both a mass and area basis was observed in this study (Fig. 7). Although we could not separate out the effects of water stress and photosynthetic  $\text{CO}_2$  drawdown in our  $\delta^{13}\text{C}$  measurements, if either variable decreases reflectance through changes in leaf morphology, this is a potential way that foliar N could affect light scattering within the leaf. Nitrogen could also be influencing cell growth within the leaf. Rademacher and Nelson (2001) found that, in tall fescue (*Festuca arundinacea* Schreb.), mesophyll tissue was the tissue fraction most responsive to changes in N. More specifically, they observed that the proportion of IAS to total mesophyll space was lower in higher N foliage because mesophyll area was enhanced by high N fertilization to a greater degree than low N fertilization. Although we did not directly measure

%IAS in this study, we could infer the number of refractive interfaces between cell walls and the intercellular airspace through the scattering coefficient (NIR reflectance/ NIR transmittance). We found that the scattering coefficient decreased as foliar  $N_{\text{mass}}$  increased, suggesting that there was a negative relationship between %IAS and  $N_{\text{mass}}$  across the species in this study. Conversely, Niinemets (1999), across a broad range of species, observed a negative relationship between density and both  $N_{\text{mass}}$  and the fraction of leaf mesophyll as intercellular airspace. This implies that leaves with high foliar  $N$  should be less dense and have a greater %IAS. Further work will be needed to tease out the relationships between internal leaf parameters,  $N$ , and radiation scattering.

Multiple regression models only improved the SW reflectance prediction by 2% (from an  $r^2$  of 0.29 with  $N_{\text{mass}}$  as the only predictor, to an  $r^2$  of 0.31 when LMA and EWT were also included in the model), and none of the leaf traits we measured explained more than 1% of the variability in the  $N_{\text{mass}}$  – SW or NIR reflectance correlations. Other studies have also observed large variation in leaf reflectance within a single species (e.g., Cochrane 2000). This suggests that factors other than those that we measured are important in determining how much light is reflected from or transmitted through leaves. General scatter around the prediction lines could be due to a multitude of different factors, including: differences in leaf maturity (Gausman et al. 1970, Carter et al. 1989), dehydration (Gausman et al. 1974b), stress/ disease (Rock et al. 1986, Knapp

and Carter 2001), or differences at the cellular level (i.e.  $A_{mes}/A$ , %IAS, cuticle thickness, etc.; Knapp and Carter 1998, Slaton 2001).

Contrary to our results, Ollinger et al. (2008, SI text) report a strong positive relationship between fresh foliage NIR reflectance and foliar N ( $R^2 \sim 0.5$ ). However, their sampling method differed from this study in that they included both conifer and deciduous species, and they measured an optically dense stack instead of single leaves using a bench top spectrometer with no integrating sphere. Only including the deciduous species during statistical analysis resulted in an insignificant relationship between NIR reflectance and  $N_{mass}$ . Perhaps the absence of a positive relationship between N and foliar reflectance in our data is simply due to our sampling design of only measuring deciduous species. Nevertheless, we did see a large range in  $N_{mass}$  even within each of the three species; if the N - canopy albedo relationship were driven primarily by N interactions at the leaf level, we would expect to see a response even within a species.

Overall, although some interesting leaf-level relationships emerged, the leaf level N - albedo relationship was the inverse of what has previously been observed at the canopy level (Ollinger et al. 2008, Hollinger et al. 2010). Additionally, stacks of multiple leaves, which simulate canopy layers without the canopy structure, exhibited the same negative relationship between reflectance and  $N_{mass}$ . This suggests that there are factors other than leaf level N-light interactions that are driving the N-albedo relationship observed from the canopy. Dungan et al. (1996) also observed no N fertilization treatment differences in

infrared reflectance at the needle level for douglas fir seedlings, although differences could be seen at the canopy level. Similarly, Knapp and Carter (1998) did not see a large difference in optical properties at the leaf level across a large range of species (and presumably a large range in foliar N).

If leaf-level interactions are not the primary influence on the previously observed N-albedo relationship, it is likely structural properties at the canopy or stand level that are the major drivers. (For a brief discussion of canopy spectral measurements from HF and ORNL, and the results from a simple modeling exercise highlighting the influence of canopy structural parameters on surface albedo see the appendix). Ollinger et al. (2008) have shown that for temperate and boreal forests, there is no relationship between canopy albedo and LAI; however there are a number of other canopy-level factors that could be influencing albedo. Ogunjemiyo et al. (2005) estimated canopy albedo and rugosity (essentially the roughness or complexity of the outer surface of a canopy) for douglas fir forests in the Cascade Range in the western US. Their results suggest that shortwave albedo declines about 3% for every 4-m increase in canopy rugosity, which they attribute to greater complexity promoting absorption of scattered light. However, rugosity is measured as the standard deviation of height in a given area (Ogunjemiyo et al. 2005), and Ollinger et al. (2008) did not see a relationship between albedo and canopy height with deciduous and coniferous species combined.

The N-albedo relationship could also stem from other within-canopy structural parameters. For example, Close and Beadle (2006) found that as foliar

N increased the leaf angle decreased (i.e. leaves became more horizontal in orientation relative to the ground) in eucalyptus seedlings. This could lead to increased canopy reflectance because it would limit the movement of uncollided or scattered radiation down through the canopy (Asner 1998). Niinemets et al. (2002) found a similar relationship between site fertility and scots pine needle inclination angle. However, results are inconsistent with two other studies (Kuers and Steinbeck 1998 and Gielen et al. 2003) where no relationship between leaf angle and N fertilization was observed for two deciduous species (sweetgum and poplar, respectively).

The area of individual leaves has also been shown to increase with increasing foliar N. Maier et al. (2008) found that N fertilization increased needle length in loblolly pine and Trapáni et al. (1999) observed an increase in leaf area in sunflowers due to increased N fertilization. Visual inspection of the sweetgum leaves used in our study support this, with N fertilized leaves appearing much larger than leaves from the ambient or elevated CO<sub>2</sub> treatments (Fig. 9). In a modeling study, Rautiainen et al. (2004) demonstrated that canopy reflectance increased as canopy volume increased; therefore, if increasing leaf area increased the canopy volume, this could be a potential link between N and canopy albedo.

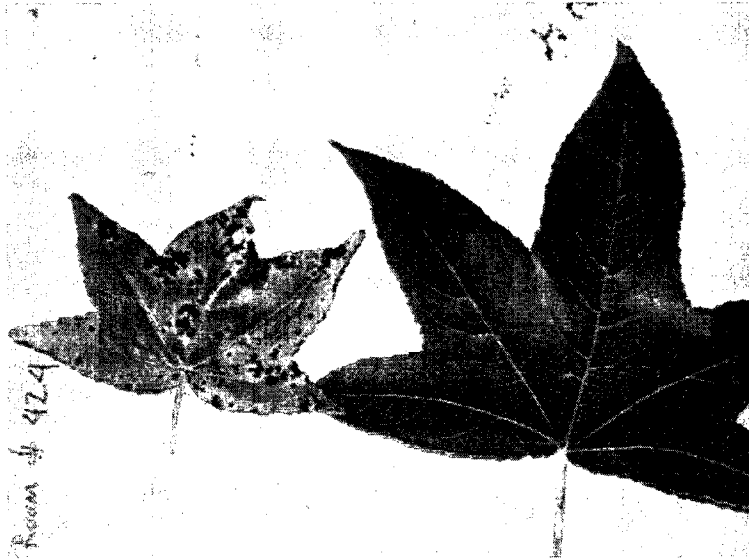


Figure 9. Sweetgum leaves collected from ORNL. On the left, a typical leaf subjected to elevated  $\text{CO}_2$ ; on the right, a typical leaf subjected to N fertilization. Although not measured in this study, visual inspection shows greater leaf area in N fertilized leaves.

It is also possible that broad-scale changes in canopy albedo are due to differences in species composition. Recent studies have shown that species classification is possible through leaf and canopy spectral indices (e.g. Martin et al. 1997, Roberts et al. 2004, Castro-Esau et al. 2006). This suggests that different species likely have distinct structural and chemical features in their leaves and canopy. Increasing our knowledge of these differences will likely improve our understanding of what causes N and albedo to covary.



## **Conclusions**

This study investigated the importance of leaf-level N-light interactions as a driver of the previously observed nitrogen- canopy albedo relationship.

Although we cannot completely rule out the importance of leaf-level N – albedo interactions, our results suggest that they are not the dominant influence on this relationship. It is likely that canopy structure, which could potentially covary with canopy N status, is the main driver of this relationship. Future work should include investigation of these canopy structural parameters in conjunction with both N and albedo in order to better understand the underlying mechanisms driving the positive correlation seen at a broad scale. An understanding of this relationship would allow us to better understand interactions in the Earth's climate system, and would improve parameterization of, and predictions from, climate and ecosystem models.

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## APPENDIX

## **Radiative Transfer Modeling**

In order to estimate the potential importance of various canopy structural parameters in influencing canopy reflectance, we used the SAIL (Scattering by Arbitrarily Inclined Leaves) radiative transfer model (Verhoef 1984, Verhoef 1985) to integrate leaf level reflectance measurements to canopy-level albedo. The SAIL model predicts the bidirectional reflectance of plant canopies using radiative transfer theory to characterize the flow of energy through a canopy. SAIL is based on the Suits model (Suits 1972), which only takes into account vertically and horizontally angled leaves. SAIL improves Suits' predictions by basing extinction and scattering coefficients on a leaf inclination angle distribution and a given LAI. This model has been discussed extensively in the literature for both crops and forests (e.g. Huemmrich and Goward 1997, Andrieu et al. 1997, Daughtry et al. 2000, Zhang et al. 2005, Zhang et al. 2006). SAIL has undergone various minor changes since its conception, and we chose to use the SAIL-2 version of this model (Braswell et al. 1996), which includes hot spot parameterization (self shading effect of both stems and leaves) (Kuusk 1991) and a two-component canopy (photosynthetically active and non-photosynthetic vegetation). Model inputs include reflectance and transmittance of component leaves, plant area index (PAI, including both photosynthetic and non-photosynthetic parts), reflectance of stem and soil substrate, mean leaf inclination angle (ellipsoidal distribution with mean leaf angle), a hot spot parameter, and viewing and solar geometry. We used a combination of measured and literature values to parameterize the model.

Sensitivity analysis of the SAIL-2 parameters was carried out first by varying each model parameter along its appropriate range individually. From the results of this simple sensitivity analysis, the parameters that most strongly influenced canopy reflectance were chosen for a Monte Carlo approach sensitivity analysis as described in Ollinger et al. (1998). The Monte Carlo approach allowed us to determine the relative importance of various canopy structural parameters in influencing canopy albedo. Multiple model runs were carried out using the spectral measurements from field observations, with LAI, mean leaf angle, fraction ground cover, and fraction of PAI due to non photosynthetic vegetation (SFRAC) determined stochastically from ecologically appropriate distribution functions. LAI was allowed to vary randomly from 1 to 8, with a mean of 5.1 and a standard deviation of 1.6, which are the values reported by Asner et al. (2003) in the global LAI distribution of temperate deciduous broadleaved forests. Mean leaf inclination angle was allowed to vary randomly from 0 to 90, with a mean of 60° for sweetgums (Kjelgren and Clark 1993) and 33° for black oaks and red maples (Hutchison et al. 1986), with a standard deviation of 10° in both cases. SFRAC was set as a normal distribution with a mean of 0.1 and a standard deviation of 0.03. Fraction cover was allowed to vary from 0.5 to 1, with a mean of 0.8 and a standard deviation of 0.1. The model was run 1000 times for each treatment, and the randomly chosen LAI, leaf angle, SFRAC, and fraction cover were recorded along with the predicted canopy albedo.

LAI and fraction cover only explained more than 10% of the variation in albedo when the understory had very different reflectance properties than the canopy. This only occurred in the ORNL ambient CO<sub>2</sub> plot, where the difference between the understory NIR and the canopy NIR values was quite substantial (around 0.5). Otherwise, leaf angle and SFRAC explained the majority of the variance in albedo (Fig. A1). On average, SFRAC explained 31% (with a range of 7 to 75%) and leaf angle explained 52% (with a range of 18 to 85%). The SAIL model describes a relatively simple canopy, and hence does not take into account some variables that could influence canopy reflectance (i.e. clumping, crown shape, species composition, etc.). Nevertheless, this modeling exercise highlights the importance of non-photosynthetic vegetation, leaf angle, and the background/ understory reflectance in determining canopy albedo. It also suggests that avenues of future study should empirically address the relationships between these canopy structural parameters, N, and albedo to determine their relative importance in N-albedo interactions.

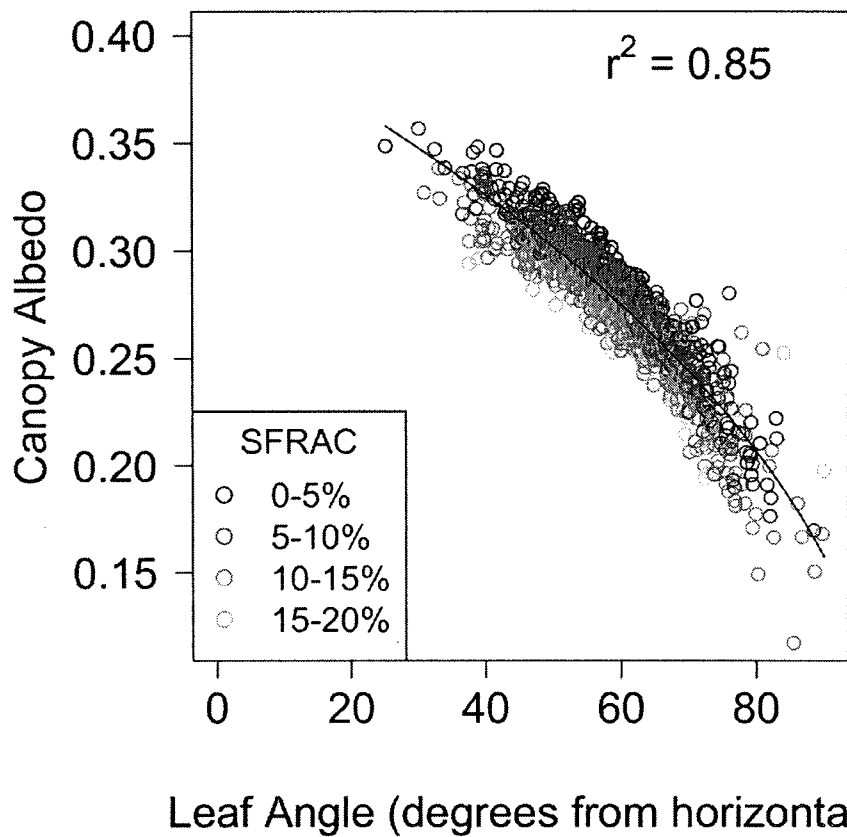


Figure A1. Results from a Monte Carlo analysis demonstrate the influence of leaf angle and the fraction of non-photosynthetic vegetation (SFRAC) on albedo values calculated via the SAIL model. Darker points correspond to lower percent SFRAC. For this run, the model was parameterized with reflectance and transmittance values of leaves from the elevated CO<sub>2</sub> treatment.



## **Canopy Spectral Images**

Canopy spectral images were acquired for the Oak Ridge FACE and N fertilized sites on July 28, 2009. Pictures were taken from a small plane with both a visible and NIR camera (both Nikon D90, one of which was retrofitted for NIR use, MaxMax LLC). AIMS (Airborne Imaging Multispectral Sensor, which includes a three-CCD multispectral camera) images were acquired over HF on August 25, 2009. The average flying height was ~ 300 m, giving an image resolution of ~ 0.16 m. Data was projected to Massachusetts State Plane Coordinate System, NAD 1983, units meter. Data was acquired and produced by the GeoProcessing Lab. of Mount Holyoke College.

From visual inspection of the Oak Ridge photos, the N fertilized plots were easily distinguishable in the visible region, but not in the NIR. The FACE plots were not easily distinguishable in either region. Furthermore, NIR differences between plots at ORNL were likely an artifact of the sun angle, as there was substantial variation in brightness across the images. For the Harvard Forest images, it was not possible to visually distinguish the fertilization treatment plots in any of the four bands (red, green, blue, and NIR). When images were resized to 5 m resolution (to decrease the high pixel-to-pixel variability) there were no significant differences between plots in any band ( $p > 0.1$ ). However, there was a significant difference in NIR, red, and blue reflectance between a nearby pine stand the mixed deciduous forest ( $p < 0.05$ , Fig. A2). These results suggest that either: differences are simply occurring at the species/ plant functional type level,

that because N fertilization started after the forest was already established we aren't seeing the changes in canopy structure that could cause an increase in reflectance, that the plot sizes are too small to detect differences due to fertilization, or that the differences in species composition and/or understory cover are obscuring the N fertilization effects. Analyzing NIR images from areas that have been fertilized with N, but are much larger in size or have been fertilized since forest conception, may help to elucidate the reason we did not observe differences between fertilization treatments.

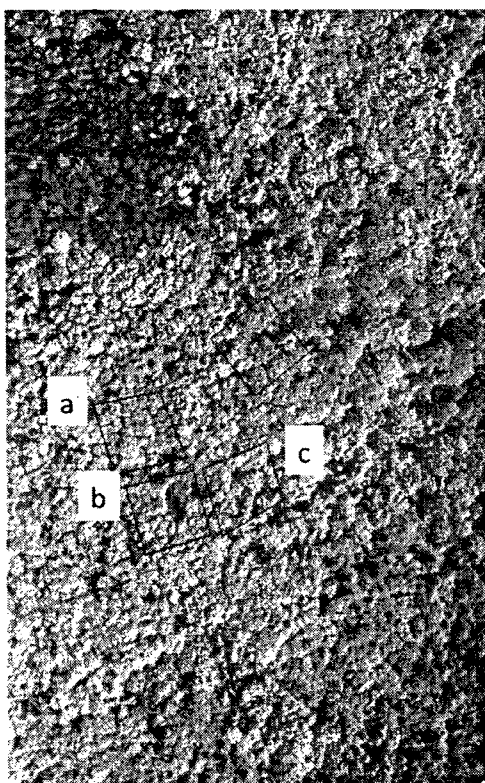


Figure A2. Harvard Forest IR image. Fertilization plots are outlined in black and labeled: (a) control, (b) low N and (c) high N. The darker pixels in the upper left are from a neighboring red pine plantation, whereas the rest of the image is mixed hardwood forest.