ENVIRONMENTAL INFLUENCES ON GAS EXCHANGE IN FERTILIZED AND NON-FERTILIZED STANDS OF LOBLOLLY PINE

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(ABSTRACT)

Spatial and temporal variation in foliar gas exchange on both a diurnal and seasonal scale was examined in 15-year-old fertilized and non-fertilized loblolly pine in the upper and lower thirds of crowns in stands located in the North Carolina sandhills. Photosynthesis rates between control and fertilized stands for both seasonal and diurnal measurement periods were different during only three months. Photosynthesis rates were consistently greater in the upper third of the crown compared to the lower third. Seasonal trends in both conductance and transpiration closely resembled trends found in seasonal photosynthesis. Foliar nitrogen concentrations were greater in fertilized stands for all months sampled. However, nitrogen content generally did not correlate with photosynthesis rates. Mean monthly water use efficiencies were significantly higher in fertilized stands during two months and were usually greater in upper crown foliage.

Common empirical gas exchange models reveal that light and vapor pressure deficit (VPD) explain a majority of the variation observed in photosynthesis and transpiration, respectively. Conductance was not modeled since environmental variation did not adequately explain conductance patterns. Predicted light response curves reveal that upper crown foliage has higher maximum photosynthesis rates, respiration rates, light compensation points, and lower initial quantum yield compared to lower crown foliage. Models predict that foliage from fertilized stands is more sensitive to VPD and light during the growing season. Transpiration models predict highly variable responses to VPD depending on the treatment combination and season. Model R^2 and predicted gas exchange values suggest that seasonal acclimation occurred.

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CHAPTER 1. INTRODUCTION AND JUSTIFICATION

Loblolly pine (*Pinus taeda*) is the major managed timber species in the southeastern United States. Productivity of these managed loblolly pine stands varies because of the intensity of common forestry practices, variation in inherent conditions that exists throughout the range of this species, and genetic variation within this species. Management practices including fertilization are known to influence gas exchange and productivity in pine. However, little stand level research has been conducted which examines spatial and temporal differences in photosynthesis and stomatal conductance and how these physiological functions are linked to changes in productivity.

Beadle *et al.* (1985) stated that it is essential to measure photosynthesis and conductance in the field to evaluate crop performance and the integrated response to habitat. Strain *et al.* (1976) similarly concluded that physiological changes in loblolly pine must be considered in order to predict net productivity. Environmental factors such as light intensity, temperature, and vapor pressure deficit guide physiological processes responsible for productivity. Therefore, determining how these variables influence gas exchange and developing response surfaces from quantitative measurements is crucial in efforts to predict long-term productivity. For example, considerably high rates of photosynthesis in slash pine through winter may significantly contribute to growth (Teskey *et al.* 1994). Since loblolly pine is generally grown in areas having mild winters, it is possible that photosynthesis and therefore carbon gain is considerable throughout the winter, but conclusive evidence does not exist because most data has been collected during the typical growing season.

Fertilization, which has been linked to increased productivity, is often an integral part of loblolly pine management. Low nutrient availability in the Southeast has resulted in the fertilization of over a million acres of loblolly pine forest (Allen 1987). Fertilization in pine stands commonly increases stem wood production, foliage production, and total biomass production (Axelsson and Axelsson 1986, Jokela and Stearns-Smith 1993, Gillespie *et al.* 1994, Albaugh *et al.* 1998). Enhanced stem wood production in fertilized stands may be the result of an increase in leaf area (Teskey *et al.* 1987, Vose and Allen 1988, Teskey *et al.* 1994) or fertilization may be directly related to

higher photosynthesis rates and therefore productivity (Mitchell and Hinkley 1993, Murthy *et al.* 1996). A study by Zhang *et al.* (1997) demonstrated that fertilization did not affect photosynthesis on a per unit leaf area in loblolly pine grown on an infertile site in Oklahoma. Tang *et al.* (1999) similarly concluded that fertilization of loblolly pine on a well-drained site in Louisiana did not significantly affect photosynthesis rates. Neither of the studies examined gas exchange throughout the entire year however. Further, multiple studies are necessary since site differences and genetic variation exist within the range of loblolly pine.

This study examines how seasonal and daily environmental conditions influence photosynthesis, stomatal conductance, and transpiration rates in both fertilized and non-fertilized loblolly pine stands. A primary objective of this study was to develop empirical models derived from gas exchange response surfaces, which will ultimately contribute to process modeling efforts by the United States Forest Service to predict the productivity of loblolly pine stands under various environmental conditions and management regimes. Process models that are developed via a scaling up process require several integrated sub-models in order to predict yield or productivity. Determining the photosynthetic capacity of loblolly pine and developing empirical relationships between gas exchange and environmental variables is therefore of primary importance since growth and yield is largely determined by potential tree carbon gain. This large collection of data also provides needed physiological information for the calibration and testing of other, previously developed process models. Finally, the gas exchange data collected for this study is perhaps one of the most extensive acquired for loblolly pine, providing critical supplementary physiological data from an already heavily studied site. These data combined with other data collected from the study site will continue to provide a wealth of information concerning the physiological ecology of managed loblolly pine forests.

The study was designed to answer several questions including the following. When is carbon gain the greatest, both on a seasonal and diurnal scale? Does fertilization influence gas exchange? What environmental variables are most highly correlated with photosynthetic rates, stomatal conductance, and transpiration rates in loblolly pine and do these variables change seasonally or with fertilization? When are certain environmental variables most limiting to photosynthesis and stomatal conductance (i.e. drought and

temperature)? Are separate empirical gas exchange models required to account for spatial and temporal differences in gas exchange? Are separate models required for fertilized stands and non-fertilized stands? Three main objectives were developed to address these questions:

1. To examine both spatial and temporal patterns in loblolly pine foliar photosynthesis, stomatal conductance, and transpiration as influenced by fertilization.

2. To determine both the spatial and temporal patterns in loblolly pine photosynthesis, stomatal conductance, and transpiration as influenced by both seasonal and daily variation in light intensity, temperature, and vapor pressure deficit.

3. To develop empirical models of the above relationships which will collectively be used for process modeling in an effort to predict the productivity of loblolly pine plantation sites.

Hypotheses related to the study objectives include:

1. Upper crown gas exchange will be greater than lower crown gas exchange.
2. Fertilized stands increase overall photosynthesis by either producing greater leaf area or increasing photosynthetic efficiency, or via both mechanisms.
3. Acclimation and the environment should be mostly responsible for seasonal variation in gas exchange while diurnal changes in the environment should explain a majority of daily variation.
4. Water limitations on hot, dry summer days may cause a reduction or even a halt in photosynthesis.
5. Models developed for both the growing and non-growing seasons should more accurately predict photosynthesis than a single model for the entire year.
6. Photosynthesis rates may be fairly similar for both the growing and non-growing seasons given the mild climate of the study location.
7. Variation in gas exchange rates among crown positions and fertilization treatments is due to both the environment and physiological differences.

CHAPTER 2. LITERATURE REVIEW

The following sections describe the effects of light intensity, temperature, and vapor pressure deficit on stomatal conductance, transpiration, and photosynthesis in the context of productivity. Although the above environmental variables are discussed in separate sections (with one exception), the dynamic interaction among these variables is discussed. The following sections also will show how photosynthesis, stomatal conductance, and transpiration intimately interact. Empirical gas exchange modeling and process modeling efforts are also reviewed.

Stomatal Conductance and Transpiration

Vapor Pressure Deficit[1] and Temperature

One of the most influential environmental variables governing stomatal conductance is the vapor pressure deficit (VPD)[2] between the saturated leaf intercellular air spaces and air surrounding the leaf. Saturation vapor pressure in the leaf is a function of leaf temperature, which fluctuates according to air temperature and radiation (Nobel 1991). Absolute humidity in the ambient air, which is also a function of temperature, is rarely at a maximum and thus some VPD almost always exists. The relationship between conductance and VPD has been demonstrated in numerous reports from various species including loblolly pine (Partake *et al.* 1998), *Yucca glace* (a prairie grass, Rosslea and Monson 1885), *Pinus sylvestris* (Scots pine, Beadle *et al.* 1985), *Elates suineans* (oil palm, Dufrene and Saugier 1993), *Vigna unguiculata* (cowpea, Bates and Hall 1982) and several others. In all cases, an increase in VPD decreased stomatal conductance. Also, it should be noted that in several of the above cases, low soil water potential coupled with a high VPD contributed to accelerated stomatal closure.

The typical stomatal response to a high VPD is thought to be the result of increasing water loss either directly from the stomata (peristomatal or epidermal hydropassive mechanism) or via complex integrated metabolic responses resulting from water stress. Transpiration will generally increase when the VPD is increased until rapid

[1] Aphalo and Jarvis (1991) concluded that water vapor saturation deficit is a more appropriate variable for describing stomatal responses to humidity.
[2] Absolute humidity deficit (AHD) is alternatively used in some papers.

water loss triggers a stress response. This change in VPD eventually causes stomatal closure, resulting in water conservation. This process improves leaf water status and will often decrease or maintain transpiration rates despite an increase in VPD. However, an increase in VPD may be enough to overcome increasing stomatal resistance, resulting in a potential increase in transpiration with increasing VPD. Assuming hypothetically that conductance is constant, an increasing VPD will directly increase transpiration since transpiration is equal to stomatal conductance multiplied by the driving force (VPD).

Little change in stomatal conductance occurred at low temperatures in Scots Pine, presumably because low temperatures were associated with a relatively low VPD (Beadle *et al.* 1985). Since the magnitude of the VPD is the primary driving force for stomatal conductance, stomatal responsiveness to VPD is often less at high temperatures and may be due to smaller fractional changes in vapor pressure relative to saturation for a determined change in vapor pressure (Beadle *et al.* 1985, Ball *et al.* 1987). In the Southeast, absolute air humidity varies both seasonally and daily depending on air temperature, evaporation sources, precipitation, and the movement of air masses (Hinckley and Braatne 1994), but it generally is much higher in the summer. VPD is usually higher in the summer since the average temperature is significantly greater than in the winter. VPD also varies spatially within the crown. Tang *et al.* (1999) showed that the upper crown foliage of loblolly pine had a significantly greater VPD, corresponding to higher stomatal conductance and transpiration values compared to lower crown foliage.

The optimization theory of Cowan and Farquhar (1977) is one of the most critically tested hypotheses regarding stomatal function. This theory states that stomatal conductance is optimal if the ratio of the change in transpiration and net photosynthesis to the changes in stomatal conductance is constant (expressed as $[\partial E/\partial g]/[\partial A/\partial g]$). Various studies have supported this hypothesis (Farquhar *et al.* 1980, Meinzer *et al.* 1984) while many, including a diurnal loblolly pine study (Fites and Teskey 1988), failed to support this hypothesis. In loblolly pine, the ratio $[\partial E/\partial g]/[\partial A/\partial g]$ generally increased diurnally and was mostly attributed to increasing $\partial E/\partial g$ and fairly constant $\partial A/\partial g$. More uniform ratios consistent with the optimization theory were exhibited on days with a more uniform environment. Seasonal trends regarding this theory have not been observed.

Species response to VPD varies, but generally leaf conductance in pines is less affected by changes in VPD compared to other tree species (Teskey and Hinkley 1986, Teskey *et al.* 1986). In a recent study, Pataki *et al.* (1998) compared canopy conductances in *Pinus taeda* and two common eastern deciduous tree species, *Liquidambar stryaciflua* and *Quercus phellos*, during mid-June in North Carolina. They concluded that *Pinus taeda* and *Liquidambar stryaciflua* had greater reductions in conductance at high water potentials due to higher VPDs than *Quercus phellos.* The rate at which conductance decreased varied as well. VPD peaked in all three species at four in the afternoon, but the diurnal sap flux peak did not correspond to maximum VPD. In *Pinus taeda*, the diurnal sap flux peaked at noon when VPD was relatively low, demonstrating an avoidance response to elevated VPD. A decrease in the diurnal sap flux of *Pinus taeda* was apparent when the VPD was about 0.4 kPa, indicating some stomatal closure increased resistance and thereby decreased flux. Stomatal conductance on both clear and overcast days decreased linearly with increasing transpiration, but exhibited decreasing sensitivity to VPD at values greater than 2 kPa. These results are indicative of a feedback mechanism in which increasing transpiration lowers leaf water potential, thereby decreasing stomatal turgor and causing stomatal closure. Bongarten and Teskey (1986) reported similar results. They found that conductance in well-watered seedlings declined from 0.40 to 0.26 cm s^{-1} as absolute humidity deficit (AHD) increased from 7 to 14 g m^{-3}, but a change of only 0.26 to 0.24 cm s^{-1} was exhibited as AHD increased from 14 to 21 g m^{-3}.

As suggested earlier, soil moisture and water potential may also influence how VPD affects conductance. This has been demonstrated in several pine species with various results. Beadle *et al.* (1985) found that in mature Scots pine, stomatal conductance remained independent of water potential despite an extended period of drought. A high AHD in white pine, had little negative affect on net photosynthesis and stomatal conductance when soil moisture was high (Maier and Teskey 1992), probably because adequate amounts of water from the soil were available and utilized as transpiration increased. Teskey *et al.* (1987) concluded that loblolly pine seedlings generally have a relatively small response to AHD regardless of soil moisture conditions. However, generally a high AHD resulted in significantly reduced net photosynthesis and

leaf conductances compared to those seedlings exposed to a low AHD. In contrast, Pataki *et al.* (1998) concluded that small decreases in soil water potential caused a reduction in loblolly pine stomatal conductance, even in relatively moist soils. Other studies have demonstrated that stomatal conductance is poorly correlated with soil water potential until a threshold is reached, in which conductance declines significantly with incremental decreases in soil water potential. Teskey *et al.* (1986) found that in loblolly pine seedlings stomatal conductance and transpiration dropped significantly at a xylem pressure potential less than –1.0 MPa (when all other environmental variables were held constant). The drop in stomatal conductance corresponded to a decrease in net photosynthesis as well.

In summary, loblolly pine conductance appears to be less affected by high VPD in comparison to other non-pine species. However, the water status of the entire tree may largely influence change in both conductance and transpiration. Therefore, changes in conductance and transpiration are probably heavily dictated by both fluctuations in VPD and plant water potential. Since VPD is higher and water potential is generally lower on warm dry days, loblolly pine probably exhibits largest changes in conductance and transpiration in the summer.

Light Intensity

Increasing light intensity generally stimulates stomatal opening by mechanisms poorly understood. Therefore, light intensity is integrally related to plant carbon dioxide and water exchange. The general belief is that stomata respond to light either indirectly, resulting from changes in C_i (internal leaf CO_2 concentration) that are directly the result of light-induced changes in photosynthetic rates (Sheriff 1979), or directly, by stimulating guard cell photoreceptors (Meinzer 1982). In general, as light levels increase, stomatal conductance increases with photosynthetic capacity, although stomatal conductance usually increases at a lesser rate than photosynthesis causing a decrease in C_i (Sage and Reid 1994). Therefore, high light intensity may produce stomatal limitations on photosynthetic rates. This response may actually be the indirect result of increasing leaf temperature due to higher irradiance and, in turn, increasing VPD. Also, the wavelength of light apparently influences stomatal aperture. Numerous studies have

concluded that both blue and red light stimulate stomatal opening subsequent to signally events initiated by photoreceptor systems (Hinkley and Braatne 1994). However, they appear to stimulate stomatal opening under different light intensities. It is believed that the blue light response by stomata is critical at low light and the red light response is more important at high light intensity. The blue light receptor is probably located in the plasma membrane of guard cells, although the actual receptor is unknown. The red light receptor appears to be chlorophyll.

Examples of stomatal responses to irradiance in conifers include a study conducted on *Pseudotsuga menziessii* saplings (Douglas Fir, Meinzer 1982). Stomatal responses to irradiance varied seasonally. During the autumn and winter, stomatal conductance was not responsive to changes in light intensity. During the summer, stomatal conductance decreased with decreasing irradiance, resulting in a constant ratio of stomatal conductance to net photosynthesis when irradiance varied. Thus, Meinzer concluded that the seasonal variation in stomatal conductance was in response to temporal changes in environmental conditions and possibly seasonal acclimation. In the winter, the stomatal behavior reflected an effort to maximize carbon assimilation rather than minimize water loss. Conversely, during the summer, the stomatal response to light appeared to correspond to the need to regulate water use efficiency rather than maximize carbon gain.

Irradiance and VPD appear to be the environmental factors having the most direct influence on water use efficiency and ultimately the role of stomatal conductance in productivity while temperature indirectly affects conductance by directly affecting VPD. Stomatal conductance is directly related to photosynthesis rates and therefore linked to productivity since the carbon available for fixation via photosynthesis (C_i) is primarily a function of the influx of carbon, governed by stomatal conductance, and carbon use, largely a function of carboxylation efficiency. Stomata function as complex multisensory structures, differentially functioning under the influence of variable light intensities and wavelengths, intercellular carbon dioxide concentrations, VPDs, soil and cellular water potentials, and hormonal signals.

Photosynthesis

Vapor Pressure Deficit

Photosynthesis is indirectly related to VPD since stomatal conductance influences internal carbon (C_i) concentrations and therefore affects the amount of CO_2 available for reduction via photosynthesis. Conifer gas exchange studies, including loblolly pine studies, have consistently shown that photosynthesis and stomatal conductance are closely coupled (Teskey *et. al* 1986, Mitchell and Hinckley 1993, Tang *et. al* 1999). Increases in VPD reduce photosynthesis by decreasing stomatal aperture and reducing $C_{i.}$ In Scots Pine, the relationship between photosynthesis and stomatal conductance was directly correlated with VPD at large deficits (Beadle *et al.* 1985). The relationship was curvilinear at a high VPD, but independent of VPD at lower deficits. VPD therefore plays a vital role in governing the resources available for carbon assimilation, which directly influences productivity. VPD is also clearly affected in parallel with other physiological processes (i.e. ion uptake and water transport) that may indirectly influence productivity (Grantz 1990).

Temperature

Higher plant growth occurs between temperatures of 0°C and 45°C, but specific temperature optimum is species dependent (Hopkins 1995). In most temperate C3 plants, photosynthesis increases with rising temperature and is optimal between 20°C and 30°C. Usually, the rate of photosynthesis sharply decreases above 30°C to 35°C. However, photosynthesis appears to shift its optima according to the temperature optimum for growth. Temperature responses of photosynthesis are also dependent on VPD as discussed earlier, since increased temperatures (when holding relative humidity constant) cause an increase in VPD. At low temperatures in Scots pine, the photosynthetic rate was virtually independent of stomatal conductance (presumably because VPD was lower) while at high temperatures, photosynthesis was strongly correlated with conductance (Beadle *et al.* 1985).

Temperature influences photosynthesis rates by affecting enzyme stability and kinetics associated with photosynthesis, and also by affecting the efficiency of the photosynthetic electron transport chain. Net photosynthesis generally increases linearly

at low temperatures. As temperature continues to rise, an optimum temperature is reached followed by a sharp drop in photosynthesis due to thermal inhibition. Net photosynthesis is directly related to the rate of respiration. Respiration has an approximate Q_{10} of two, indicating a logarithmic increase with temperature. In most plants, respiration is not inhibited until temperatures of 40 to 45°C while gross photosynthesis peaks between 20°C and 30°C in C3 plants. Therefore decreased net photosynthesis at high temperatures is largely due to a disproportionate increase in respiration relative to gross photosynthesis. Photosynthesis rates at high temperatures also depend on thermal properties of photosynthetic enzymes, thylakoid membrane stability, and the thermal stability of the photochemical reactions. The thermal inhibition of key enzymes has been shown to correspond exactly to the thermal inhibition of photosynthesis, but this appears to vary among species (Nilsen and Orcutt 1996). Photosystem II (PSII) inhibition at high temperatures also closely corresponds to photosynthesis inhibition in some species. At temperatures below the optimum, enzymes associated with carbon fixation reactions including rubisco, fructose-bisphosphatase, and phosphotase have been implicated as rate-limiting enzymes. Low temperatures also cause the down-regulation of enzymes and electron carrier complexes associated with the light reactions including NADP reductase, plastocyanin, cytochrome F1, and ATP synthase. Indirectly, low temperatures may cause photoinhibition since PSII is unstable at suboptimum temperatures associated with excessive light energy. At low temperatures and high irradiance, an excess of photon energy coupled with the low enzymatic rates overexcites the PSII complex (since more photon energy is being supplied than can be utilized). This causes the dysfunction of the PSII complex, ultimately leading to photo-oxidative damage and the degradation of chlorophyll. Thus, temperature effects on photosynthesis are clearly directly related to the activity and functionality of enzymes and protein complexes associated with both the light and dark reactions.

Plant productivity is clearly affected, both directly and indirectly, by photosynthetic responses to temperature. Temperature affects productivity by (1) limiting the extent of the growing season and the amount of diurnal growth (2) controlling the rate of dark respiration, (3) affecting the rate of photorespiration, and finally (4) influencing the rate of photosynthesis. An example of an indirect response of

photosynthesis to temperature is apparent in a case where high temperatures in Scots pine and European larch resulted in increased chlorophyll production while low temperature favored root dry matter accumulation (Gowin *et al.* 1990). This may be a long-term response to both storage needs during the colder winter and increased photosynthesis requirements during the warmer growing season. A more direct example is apparent in *Gossypium hirsutum* L, where suboptimum leaf temperatures were linked directly to reduced photosynthesis and dry matter production when combined with high light intensity (Winter and Koeniger 1991).

Strain *et al.* (1976) working with loblolly pine determined that temperature, in part, influenced maximum photosynthesis rates both seasonally and daily. The temperature corresponding to maximum net photosynthesis varied seasonally. The field study, which was conducted in North Carolina, determined that the approximate temperatures of maximum net photosynthesis were 25°C in June, July, August and September, but only 10°C in December and January. These values are consistent with data later published by Drew and Ludig (1981). Net photosynthesis rates were the highest in May and were decreasingly lower in July, November, and January. This demonstrates the potential for seasonal acclimation to climatic conditions, but it does not explain what other environmental variable or variables contributed to the response. Strain *et al.* (1976) also concluded that daily variation in temperature drastically affected net photosynthesis rates. In the spring, photosynthesis dropped quickly at temperatures above 20°C while in the summer rates dropped rapidly after 30°C. In contrast, the winter net photosynthesis rates, although much lower than peak summer or spring rates, were fairly constant over the range of 0°C to 15°C. This suggests that while photosynthesis may drop significantly (or even virtually cease) throughout the day during the summer and spring, winter photosynthesis may be relatively stable throughout much of the day (when all other environmental variables are held constant). Therefore large amounts of carbon might be fixed over the course of a winter day – especially a relatively mild, sunny day. If this is true, significant carbon accumulation (i.e. yield) could occur in the winter. Perry (1971) observed winter growth in loblolly pine seedlings on a plantation site near Raleigh, North Carolina. The seedlings increased in average weight by over 30% from November through February. Other studies have shown that photosynthesis in

loblolly pine does not respond to a wide range of temperatures under experimental conditions. Teskey *et al*. (1986) reported that temperatures ranging from 20°C to 35°C had a moderate affect on photosynthesis in loblolly pine seedlings compared to other environmental variables, resulting in about a 25% reduction in mean photosynthesis at 35°C relative to the highest mean rate which occurred at 20°C.

Although the northern range of loblolly pine may be restricted partly by low temperatures in the winter, the southern range may be as limited by high temperatures that restrict photosynthesis during the summer months. In the northern limits of the range, frost may also limit photosynthesis. The frost-free period varies from over 300 days in the southern range to around 180 days in the northernmost limits. Even if the frost is restricted to the nighttime, photosynthesis the following day sometimes does not reach significant levels, presumably due to a reduction in maximum leaf conductance (Teskey *et al.* 1987).

Light Intensity

Light intensity changes throughout the day and year as the result of the dynamic movement of the sun, and changes in weather and atmospheric composition. In tree species, light levels also vary depending on the location of the foliage in the crown, the density of the crown foliage, and the extent of crown closure in the forest or the stand. Light has three general effects on photosynthesis that are critical to function and productivity. Light provides the energy used for the production of ATP and NADPH. It also activates critical enzymes involved in photosynthesis and, as previously discussed, light stimulates stomatal opening. Light also indirectly stimulates the development of leaves and photosynthetic apparatus (Sage and Reid 1994).

In loblolly pine, light-saturated (photosynthetic photon flux density > 1600 μmol m^{-2} s^{-1}) net photosynthetic rates (A_{sat}) varied throughout the year (Murthy *et al.* 1997). The rates peaked in April and were lowest in January and September. Although water limitations in the summer could have reduced photosynthesis, irrigation had no significant influence on $A_{sat.}$ However, nutrient additions to the soil did have a significant effect on $A_{sat,}$ which increased with the addition of nitrogen. Another loblolly pine study found that upper crown levels had significantly greater photosynthetically active radiation

(PAR) levels, which corresponded to higher photosynthesis rates in comparison to lower crown foliage (Gravatt *et al.* 1997). Within crown variation in photosynthesis was greatly dependent on canopy light levels. The same study also determined that a significant time-of-day effect occurred at the different canopy levels. The lower crown class had the highest photosynthetic photon flux density in the early afternoon when sunlight was most direct. Increased spacing between trees also generally corresponded to an increase in lower crown mean photosynthesis, stomatal conductance, and transpiration rates. Consistent with other studies, light availability and needle conductances were positively correlated with net photosynthesis. This is consistent with work by Tang *et al.* (1999), who reported similar findings in another loblolly pine study. The study determined that upper crown foliage had significantly greater light levels and higher photosynthesis rates compared to the lower third of the crown.

Differences in the photosynthetic capacity of the upper crown and lower crown are also attributable to structural and physiological differences in sun and shade foliage. Light-response curves for several species reveal common differences between sun and shade foliage with respect to photosynthesis rates. Shade leaves generally have lower light compensation points, lower dark respiration rates, higher quantum efficiencies, lower light saturation levels, and lower maximum photosynthetic rates compared to sun foliage. Morphologically, shade leaves are generally larger, thinner, and have a thinner cuticle than sun leaves. Shade leaves are physiologically adapted to maximize the amount of light intercepted by scattering absorbed light and investing structurally in proportionally larger amounts of light harvesting complex II (LHCII) per photosystem II (PSII) via a decrease in the chlorophyll a/b ratio. Conversely, sun leaves are relatively thick and are morphologically composed of long palisade cells that focus light energy. Sun leaves maximize light energy utilization by investing in electron transport chain proteins. Thus, shade leaves attempt to maximize light energy consumption while sun leaves invest in machinery needed to utilize high (light) energy inputs.

Differences in shade and sun foliage from loblolly pine have been documented (Nowak 1991, Nowak *et al.* 1991). Under full light conditions, average net photosynthesis was significantly greater in the upper crown foliage (2.81 μmol m^{-2} s^{-1}) compared to the lower crown foliage (2.33 μmol m^{-2} s^{-1}) (p=0.06). However, no

differences in transpiration or needle conductance existed between the upper and lower crown foliage. Upper crown foliage had significantly higher net photosynthesis rates under light saturating conditions than lower crown foliage during August and October ($p<0.1$), but not during May, June, July, September, or November measurements. Predicted dark respiration was also significantly higher in the upper crown for the growing season. Differences in actual chlorophyll content, predicted quantum yield, and predicted light compensation points were not significant for the growing seasons however.

Zhang *et al.* (1997) demonstrated that leaves of seven-year-old loblolly pine acclimated to low light when artificially subjected to shade. Physiological and morphological properties of leaves acclimated to low light were similar to several of the characteristics of shade leaves outlined above. Shade acclimated foliage had a decrease in maximum net photosynthesis, stomatal conductance, specific leaf weight, N content per fascicle, and chlorophyll a/b ratio and an increase in total chlorophyll. Nutrient additions did not increase photosynthesis on a unit leaf area basis in shade acclimated foliage. This suggests that increases in leaf area from fertilization treatments may actually reduce photosynthesis on a per unit leaf area in the lower canopy since greater shading from the upper crown is likely.

The available light energy clearly influences photosynthesis rates under ambient conditions more directly than any other environmental variable. Several non-linear empirical models have been developed which predict photosynthesis rates based on only light intensity (Charles-Edwards 1981; Lieth and Reynolds 1987; Hanson *et al*. 1988). Therefore, not surprisingly the amount of light energy intercepted by a photosynthetic surface most directly correlates with productivity in several crop species (Hay and Walker, 1992). Therefore, light energy is considered the primary driver of photosynthesis and productivity. Since extensive variability in both light quality and intensity exists, carbon gain fluctuates considerably both on a spatial and temporal scale.

Seasonal Variation vs. Diurnal Variation in Gas Exchange

Gas exchange responses to VPD, light intensity, and temperature vary both within days and among seasons. Long-term physiological adjustments occur over greater

periods of time and are responsible for seasonal changes in optimal environmental conditions and maximum photosynthesis rates previously mentioned. The environment may only partly influence the shift in maximum gas exchange rates and optimums since genetics and other physiological processes may directly and indirectly affect gas exchange. Diurnal variation in gas exchange is most likely due to immediate environmental changes since short-term acclimation probably does not affect photosynthesis optimums.

Seasonal variation in gas exchange may also be due to physiological processes affecting photosynthesis that are independent of the ambient environment. Boltz *et al.* (1986) showed that net photosynthesis per unit leaf area of seedlings varied over a period from the middle of August through December even though measurement conditions (i.e. environmental conditions in the measurement chamber) were identical throughout the study. Net photosynthesis was lowest at the end of September presumably because respiration was relatively high due to peaking leaf growth. At the end of October, net photosynthesis increased by about 50% on average since foliar growth ended and respiration concurrently declined.

Empirical Gas Exchange Models and Process Modeling Efforts

Numerous empirical gas exchange models have been developed, serving as useful predictive models and providing basic information concerning plant responses to variable environmental conditions (Charles-Edwards 1981, Lieth and Reynolds 1987, Hanson *et al*. 1988, Van Wijk *et al.* 2000). Empirical models developed from plant physiological response surfaces are also essential components of mechanistic process models which incorporate multiple, integrated sub-models into a single, more complex predictive model. Process models have proven to be essential tools for predicting the integrated response of individual trees and ultimately the stand to environmental changes over both time and space (Falge *et al*. 1997). Since plant growth is determined by a number of physiological processes at several levels of organization and complexity, process modeling provides an extremely useful means for predicting highly integrated physiological processes including tree development and stand productivity (Landsberg 1986). Process models have practical uses in forest management including simulating

growth over a range of environments, site conditions, and common stand management practices such as thinning, irrigation, and fertilization.

Although a qualitative and quantitative understanding of multiple processes is necessary for estimating tree growth and yield using process models, photosynthesis responses are of primary importance since gas exchange determines potential tree carbon gain. Two types of process models have been developed for estimating whole canopy carbon and water fluxes. The first type involves a scaling-up process based on data collected from foliar gas exchange analysis, canopy structure, and light interception. The second type is referred to as the "big-leaf" model, which is derived from observations made at the whole canopy level, and describes fluxes according to principles understood at the single leaf level. Big-leaf models are generally based on simplified physiological assumptions of the "average" leaf and are usually not as accurate as models that are developed from scaled up physiological data. Models scaled up from the physiological level however require intensive data collections, which may not be practical or necessary depending of the objective of the model.

Falge *et al*. (1999) recognized the need for comprehensive information regarding functional relationships between leaf carbon assimilation and the environment for use in process model development. Along with net photosynthesis, several integrated sub-models specific to a given species and related to productivity are also necessary including those predicting carbohydrate production, carbon partitioning, nutrient uptake, respiration, and energy interception (Landsberg 1986). Also, multiple models for a single process may be necessary since acclimation may occur temporally and because spatial differences exist in the crown. For example, seasonal acclimation and changes in maximum photosynthesis rates may warrant two separate models – one for the growing season and another for the non-growing season. Likewise, since different physiological response surfaces commonly exist in sun and shade foliage, two separate models for the upper third and lower third crown positions may be required. Multiple models may further be needed to account for forestry management practices such as fertilization and irrigation since these practices influence physiological processes. Process models are therefore potentially complex and require multiple sub-models that interact and are individually reduced to explain seasonal and temporal variation.

In this study, empirical gas exchange models were developed for loblolly pine to provide necessary sub-models for a process model currently being developed by the U.S. Forest Service. This data, when integrated with the necessary sub-models mentioned above, will aid in predicting productivity of managed loblolly pine stands.

CHAPTER 3. MATERIALS AND METHODS

Study Site

All measurements were taken in Scotland County, North Carolina (35°N lat., 79°W long.) at the United States Forest Service (USFS) Southeastern Forest Tree Experiment and Education Site (SETRES). SETRES consists of 14-year-old (planted in 1985) hand planted loblolly pine stands (2 x 3 m spacing) on flat, infertile, excessively drained, sandy, siliceous, thermic Psammentic Hapludult soil (Wakulla series). The average annual precipitation is 121 cm, but drought is common in the summer and early fall. The average summer temperature is 26°C and the winter average is 9°C. The average annual temperature is 17°C. The established site study design is a 2 x 2 factorial combination of fertilized and irrigated additions replicated four times (for a total of 16 plots). The plots consist of 30 x 30 m measurement plots within 50 x 50 m treatment plots. Interaction among below ground matter from adjacent plots is prevented by a 150 cm deep plastic liner that separates plots. Non-pine vegetation is controlled by mechanical and chemical (glyphosate) treatments. No understory vegetation exists.

Nutrient applications began in March 1992 and continued through March 1998. The total amount of each nutrient (in lbs. ac^{-1}) added during that time is as follows: N (693), P (135), K (301), Ca (150), Mg (146), S (186), and B (3.5). Measurements later determined that the foliar nitrogen concentrations with respect to total nutrient concentration of leaves from fertilized plots were about .5% higher than leaves from the control plots. In the fertilized plots, crown closure is common and foliage is generally denser compared to control trees. Albaugh *et al.* (1998) found that total biomass accumulation at SETRES increased 91% with fertilization (compared to only 29% with irrigation). Since fertilization clearly has a highly significant effect on productivity and irrigation has a lesser effect at SETRES, both seasonal and daily patterns of photosynthesis, stomatal conductance, and transpiration were measured in fertilized and control plots.

Seasonal Variation in Gas Exchange

Gas exchange measurements were taken monthly from March 1999 to March 2000 at SETRES using the LiCor 6400 Portable Photosynthesis System (LiCor, Lincoln, NE). Measurements were taken using cut foliage from the upper and lower third of crowns from a subsample of 2 trees per treatment/block combination for a total of 32 measurements (2 treatments [control and fertilized] x 4 blocks x 2 crown positions x 2 subsamples). Gas exchange was measured in each block (containing both treatments) sequentially, and subsamples from each level within each treatment were chosen randomly for sampling. Blocks were always measured in the same order. This sequence was repeated two more times on the day of measurement in order to capture an abbreviated diurnal response to daily environmental changes. Measurements included morning, afternoon, and late afternoon measurement periods. A total of 96 measurements (three sampling sequences) were generally taken throughout the day. Rain on the December measurement day prevented a morning measurement period.

Shoots were cut using a pole pruner and measurements were taken immediately on a detached fascicle. All measurements were taken at the ambient temperature and humidity, and CO_2 concentrations were held constant in the chamber at 350 ppm. The average photosynthetically active radiation (PAR) was estimated for the upper and lower third of crowns and kept constant for each crown level in the block throughout a measurement period (32 measurements). The PAR for each crown level was determined by evaluating the average PAR in full sunlight (for the upper third) and the average PAR in the understory (for the lower third) prior to the measurement period. The PAR levels for both control and fertilized stands were equally assigned for a given crown position despite slight differences in PAR levels in the understory (due to leaf area differences). The PAR was reassessed and adjusted for each measurement period according to the PAR levels immediately prior to sampling. Water potentials were determined for the same branch as the sample immediately after being cut using a field pressure chamber (PMS instrument Co., Corvallis, OR). Needle diameter was immediately recorded and leaf area was later determined using the following equation (Ginn *et al.*1991):

$$LA_1 = (n * l * d) + (\pi * d * l)$$

where l = the length of the needle, d = fascicle diameter and n = number of needles in the fascicle. For the analysis purposes, values were later adjusted to represent gas exchange on a per leaf area basis. All measurements were completed in one day. Diurnal environmental variation among blocks was controlled by measuring one block at a time in the shortest amount of time required (about 1.5 hours per four blocks).

Ideally, measurement days were chosen to capture a 'typical' seasonal day, representative of the environmental conditions during that month. Within each season, environmental variation was desired in order to obtain the entire realm of possible environmental conditions that might influence photosynthesis, stomatal conductance, and transpiration.

Daily Variation in Gas Exchange

Daily environmental influences on gas exchange were examined during two intensive measurement periods taken during July 1999 and January 2000. Two consecutive days were chosen to represent the growing season (July) and the non-growing season (January). Measurements were taken hourly using cut foliage from the upper and lower third of crowns from a subsample of two trees per treatment/block combination for a total of 32 measurements per hour (2 treatments [control and fertilized] x 4 blocks x 2 crown positions x 2 subsamples). Gas exchange was measured for two subsamples simultaneously using two LiCor 6400 Portable Photosynthesis Systems. Gas exchange measurements were collected from each block sequentially and always in the same order. Subsamples from both canopy levels within the block were measured in a random order. All measurements were taken at ambient temperature and humidity, and CO_2 concentrations were held constant in the chamber at 350 ppm. PAR was controlled as described in the previous section. Gas exchange was recorded for a total of ten measurement periods over two consecutive days in both July and January. Measurements were taken from dawn until dusk for one day (six measurement periods) during both months. Water potentials for each sample were also recorded as described in the previous section. Gas exchange data were adjusted on a leaf area basis as described in the previous section.

Collection of data over the entire day for two consecutive days assured that measurements included a broad range of environmental variables representative of daily variation during the given month. Measurements captured a broad range of temperatures, cloud cover, humidity, and rainfall in a relatively short period of time. This allowed for the examination of the daily, short-term variation and diurnal variation of photosynthesis, conductance, and transpiration.

Statistical Analysis and Use of the Data

The data were analyzed as a split-block design in which the whole plot is the fertilization treatment and the split-block is the crown position (upper and lower thirds). The whole plot treatments were randomized across blocks while split-block treatments could not be randomly assigned since crown position is spatially fixed.

Treatment and position differences in gas exchange, water use efficiency, and foliar nitrogen content were statistically examined using analysis of variance. Two trees were subsampled for gas exchange measurements and the average was used as the experimental unit. Foliar nitrogen percentages were obtained from pooled needle samples collected from each block/fertilization/crown position combination every sampling month. Samples consisted of foliage for which gas exchange was measured. The following analysis of variance table was used:

Factor	*Degrees of Freedom*
Fertilization Treatment (F)	1
Block (B)	3
Whole plot error (F x B)	3
Crown position (C)	1
Split-block error (C x B)	3
Interaction (F x C)	1
Error (C x F x B)	3

The response of gas exchange to environmental variables was modeled using multiple linear regression techniques. Non-linear regression analysis was attempted

using several previously developed models; however linear regression analysis using transformed data yielded statistically more competent models. Preliminary gas exchange models were developed and compared using the stepwise procedure in SAS (SAS Statistical Institute, Cary, NC). Common simplified gas exchange models for all fertilization treatment and crown position combinations were developed based on common significant parameters. Models were developed for the entire year, the growing and non-growing seasons, and both the January and July intensive measurement periods. Differences in model parameters were then compared statistically using the common models and indicator variables. All statistical analysis was performed using SAS.

CHAPTER 4. RESULTS

Seasonal Trends in Gas Exchange

Photosynthesis

Mean monthly net photosynthesis was examined in both control and fertilized stands and the upper and lower thirds of crowns (Figure 1). Fertilized stands had significantly higher photosynthesis rates during the month of February ($p=0.09$) while control stands had significantly higher rates during April ($p=0.02$) and May ($p=0.05$). Although these are the only months in which a significantly different mean photosynthesis rate existed between treatment plots, means indicate a seasonal pattern may be occurring. Mean photosynthesis rates in the control stands were greater during most of the growing season (March to August). Mean photosynthesis varied little from September to December, but was greater in the fertilized stands during January and February. Monthly photosynthesis rates varied from approximately 1.5 $\mu mol\ m^{-2}\ s^{-1}$ in November and December to around 3.5 $\mu mol\ m^{-2}\ s^{-1}$ in March for both fertilization treatments.

Photosynthesis rates in the upper third of crowns were always significantly greater than rates in the lower third of the crowns. This trend is primarily due to differences in light levels between the upper and lower third of crowns (see "Modeling Gas Exchange"). Mean monthly net photosynthesis in the lower thirds of crown varied from slightly less than 1 $\mu mol\ m^{-2}\ s^{-1}$ in June to about 2.75 $\mu mol\ m^{-2}\ s^{-1}$ in March; upper crown photosynthesis ranged from about 2 $\mu mol\ m^{-2}\ s^{-1}$ in November and December to 4.5 $\mu mol\ m^{-2}\ s^{-1}$ in March.

Interaction between fertilization treatments and crown positions were only significant for two months, and consistent trends were not apparent.

Foliar nitrogen concentrations were determined for pooled measurement needles from a given block/fertilization treatment/crown position for eight months (Figure 2). Foliar nitrogen concentrations were always significantly higher in fertilized foliage ($p<0.1$). Mean foliar nitrogen contents were generally greater in the upper crown foliage. However, upper crown foliar nitrogen contents were only significantly greater for the months of July and October ($p<0.01$).

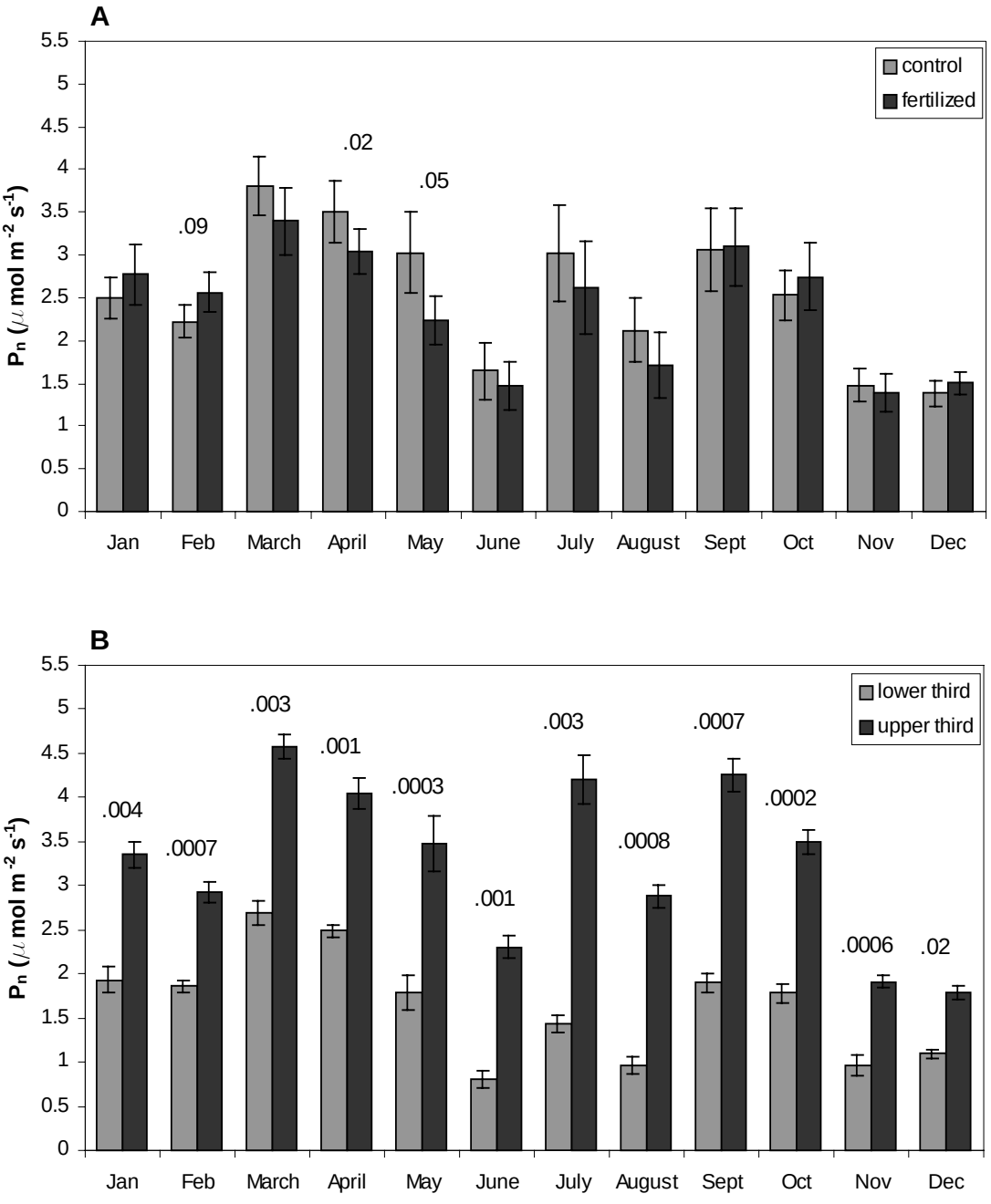

Figure 1: Mean monthly net photosynthesis rates for control and fertilized stands (A) and upper third and lower third of crowns (B). Bars indicate the standard error. Numbers above bars are p-values indicating significance levels between fertilization treatments or crown positions within months. P-values are only given when $p<0.1$. Each value is an average (n=16) from three measurement periods throughout a single day, except for December when morning measurements were not used in the analysis because of rain.

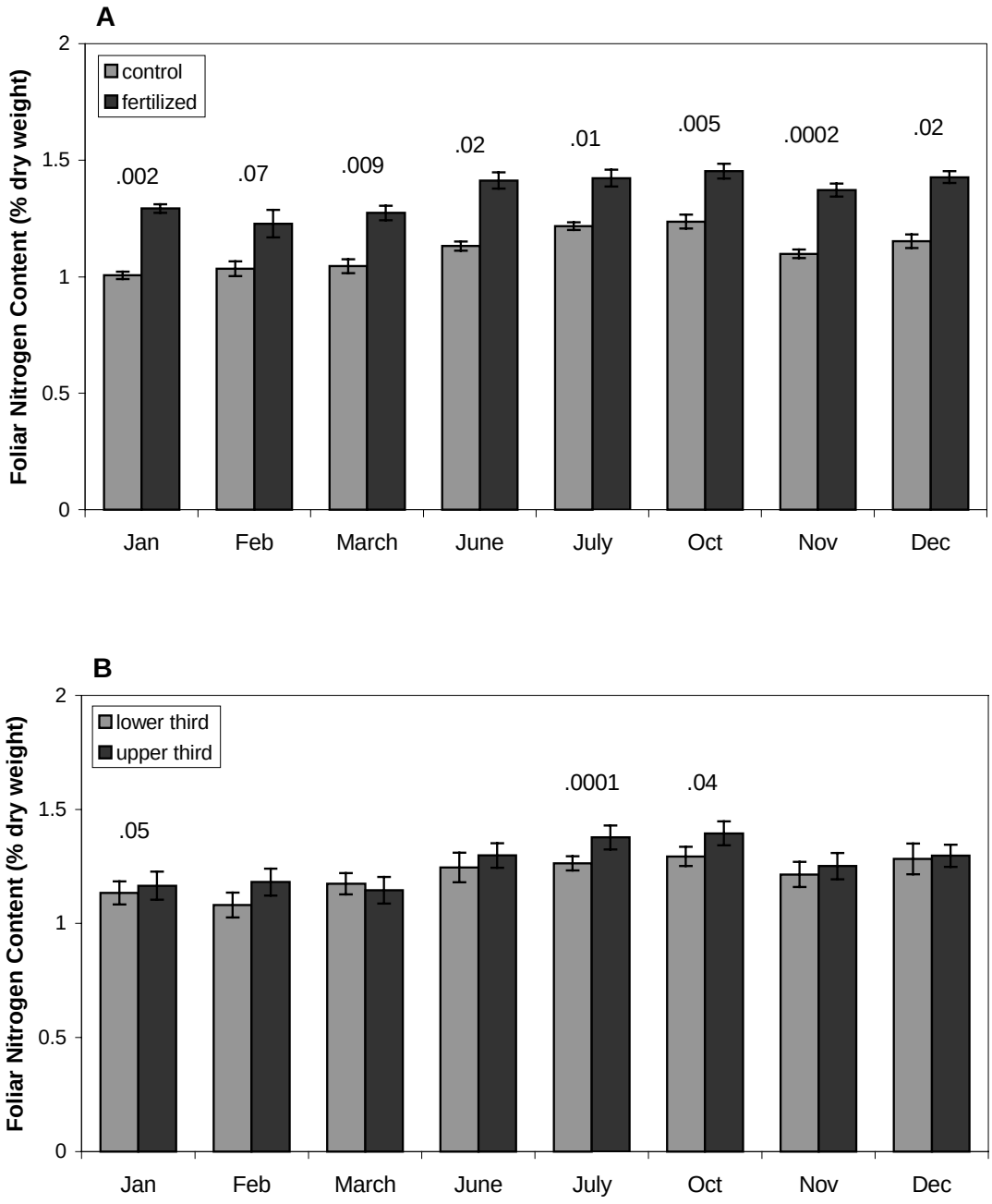

Figure 2: Mean monthly foliar nitrogen concentrations for control and fertilized stands (A) and upper third and lower third of crowns (B). Bars indicate standard error. Numbers above bars are p-values indicating significance levels between fertilization treatments or crown positions within months. P-values are only given when $p<0.1$. Values are averages (n=8) from pooled foliage in which photosynthesis rates were recorded.

Conductance

Conductance rates between fertilization treatments and crown positions were also compared statistically (Figure 3). Patterns in conductance generally closely resembled trends occurring in seasonal photosynthesis outlined in the previous section. Generally when photosynthesis rates were high relative to other months, conductance rates were also elevated. Conductance rates in control and fertilized stands mirrored seasonal photosynthesis trends. Mean conductance was significantly greater in fertilized stands during February ($p=0.008$), which coincided with higher photosynthesis rates. Conductance rates were significantly greater in control stands from March to June ($p \leq 0.05$). Mean conductance rates differed among months in the control stands, ranging from less than 0.03 mmol m^{-2} s^{-1} in August to almost 0.06 mmol m^{-2} s^{-1} in March, April, and November. Fertilized stands had a similar range of mean monthly rates throughout the year, with lowest rates slightly greater than 0.02 mmol m^{-2} s^{-1} and higher rates reaching almost 0.05 mmol m^{-2} s^{-1} during September, October, and November[3].

Conductance was significantly greater in the upper third of crowns compared to lower third for all months except November and December ($p<0.1$). Again, this trend is closely reflected in the photosynthesis rates. Mean monthly rates ranged from less than 0.02 mmol m^{-2} s^{-1} during August to greater than 0.04 mmol m^{-2} s^{-1} in March, April, and November in the lower third of crowns. In the upper third of crowns, conductance was as low as 0.035 mmol m^{-2} s^{-1} in June and August and reached almost 0.06 mmol m^{-2} s^{-1} in September, October, and November.

Interaction between fertilization treatments and crown positions occurred during January, April, May, June, and July ($p<0.1$). The interaction can be explained by the fact that differences in mean conductance rates between control and fertilized stands varied more in the upper crown foliage than in the lower crown. For example, in January the percent difference in conductance rates between fertilization treatments for the lower

[3] Conductance and transpiration trends outlined exclude December since rain occurred in the morning prior to measurements resulting in inaccurate data. All conductance and transpiration rates less than zero were not used in statistical analysis and morning data from both December and November was also excluded, resulting in 64 total measurements or less during the day (rather than 96 total measurements for a complete day).

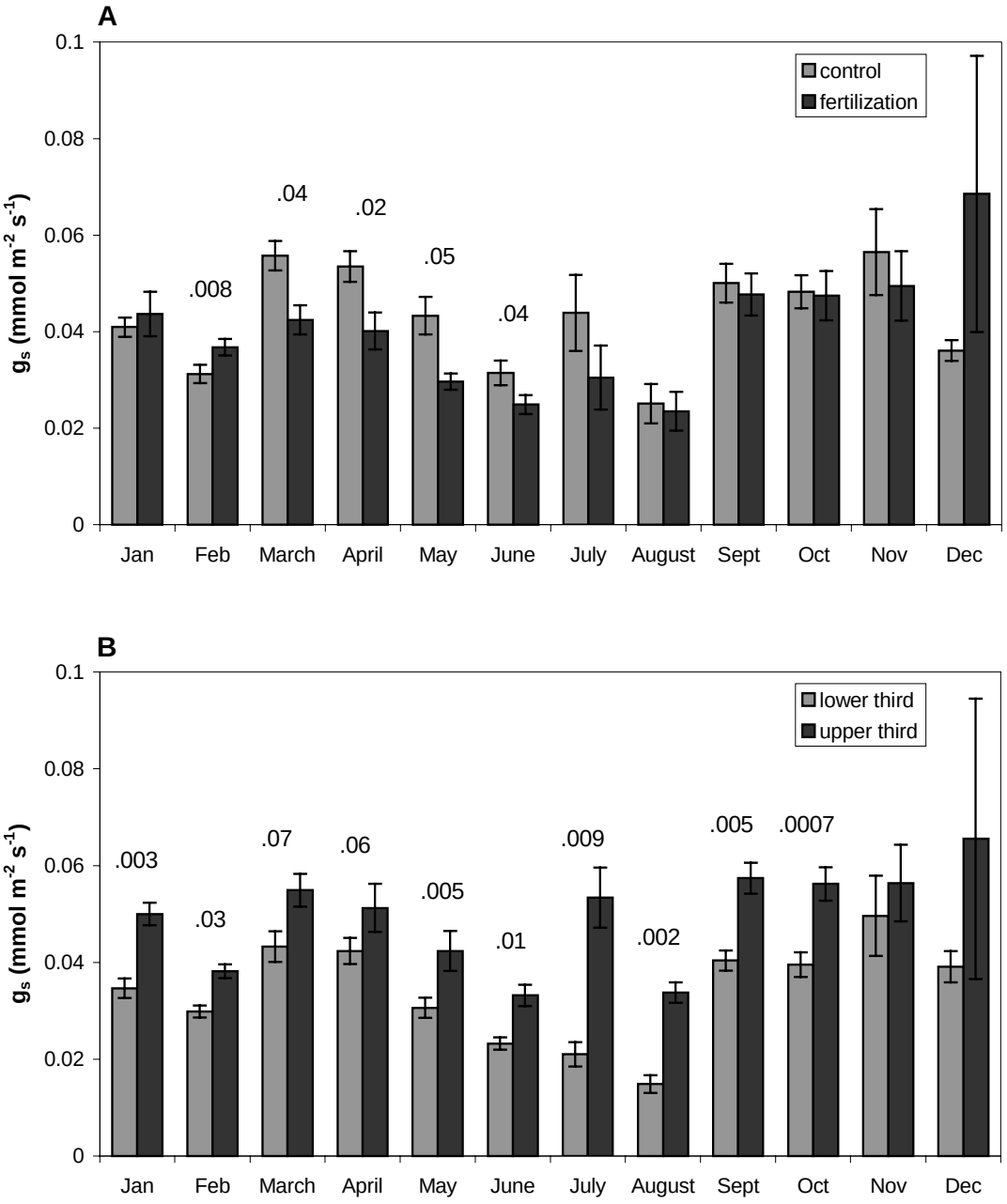

Figure 3: Mean monthly conductance values for control and fertilized stands (A) and upper third and lower third of crowns (B). Bars indicate the standard error. Numbers above bars are p-values indicating significance levels between fertilization treatments or crown positions within months. P-values are only given when $p<0.1$. Each value is an average (n=16) from three measurement periods throughout a single day, except for November and December when morning measurements were not used in the analysis because of rain.

crown position was about 11% while the percent change in conductance rates between fertilization treatments for the upper crown position was approximately 20%.

Transpiration

Statistical comparisons of monthly mean transpiration between fertilization treatments and crown positions revealed similar seasonal trends to both photosynthesis and conductance (Figure 4). Transpiration was significantly greater in fertilized stands during February ($p=0.02$) and greater in control stands in March, April, and May ($p<0.05$). Mean transpiration was generally higher in the control plots during the growing season, although means did not always significantly differ. Means in the control plots varied from around 0.25 mmol m^{-2} s^{-1} in February and June to greater than 1.0 mmol m^{-2} s^{-1} in April and May. Transpiration in fertilized stands ranged from near 0.25 mmol m^{-2} s^{-1} in February and June to slightly greater than 0.75 mmol m^{-2} s^{-1} in April and May.

As with conductance, transpiration was greater in the upper third of crowns compared to the lower third of crowns for all months except November and December ($p<0.05$). Transpiration in the lower third of crowns ranged from about 0.25 mmol m^{-2} s^{-1} in February, June, and December to greater than 0.75 mmol m^{-2} s^{-1} in April and May. In the upper third of crowns, rates varied from approximately 0.4 mmol m^{-2} s^{-1} in February, June, and December to greater than 1.0 mmol m^{-2} s^{-1} in April and May.

Interaction between fertilization treatments and crown positions was significant during four of the five same months as conductance interactions occurred – January, April, May, and June ($p<0.01$). The explanation for interaction is the same as described in the previous section and is attributed to greater changes in transpiration between control stands and fertilized stands in the upper third of crowns compared to the lower third of crowns.

Water Use Efficiency (WUE)

Water use efficiencies were compared between fertilized and control stands, and lower and upper crown foliage (Figure 5). Water use efficiencies were generally higher in fertilized stands, but were only significantly greater for the months of March and July

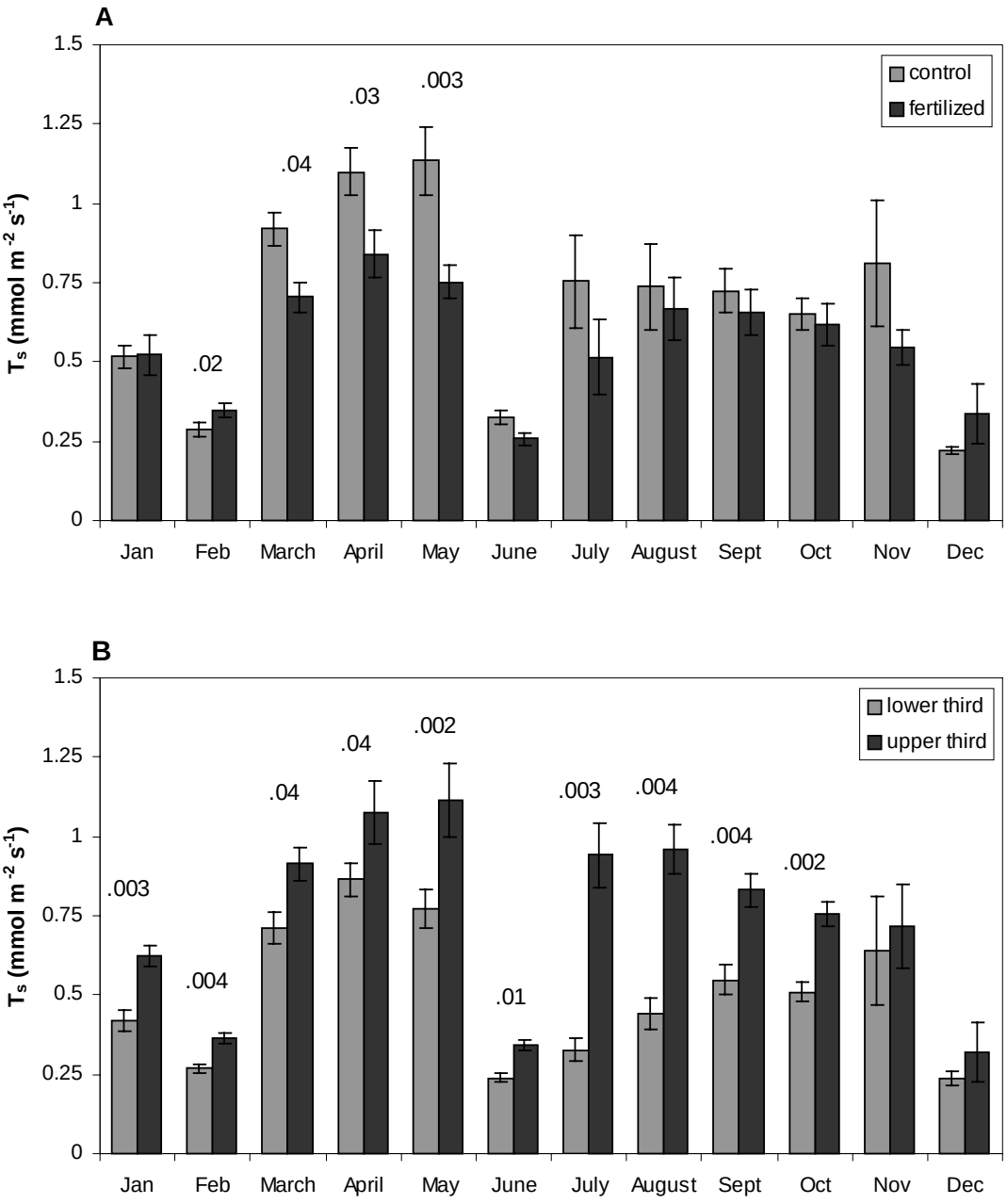

Figure 4: Mean monthly transpiration values for control and fertilized stands (A) and upper third and lower third of crowns (B). Bars indicate the standard error. Numbers above bars are p-values indicating significance levels between fertilization treatments or crown positions within months. P-values are only given when $p<0.1$. Each value is an average (n=16) from three measurement periods throughout a single day, except for November and December when morning measurements were not used in the analysis because of rain.

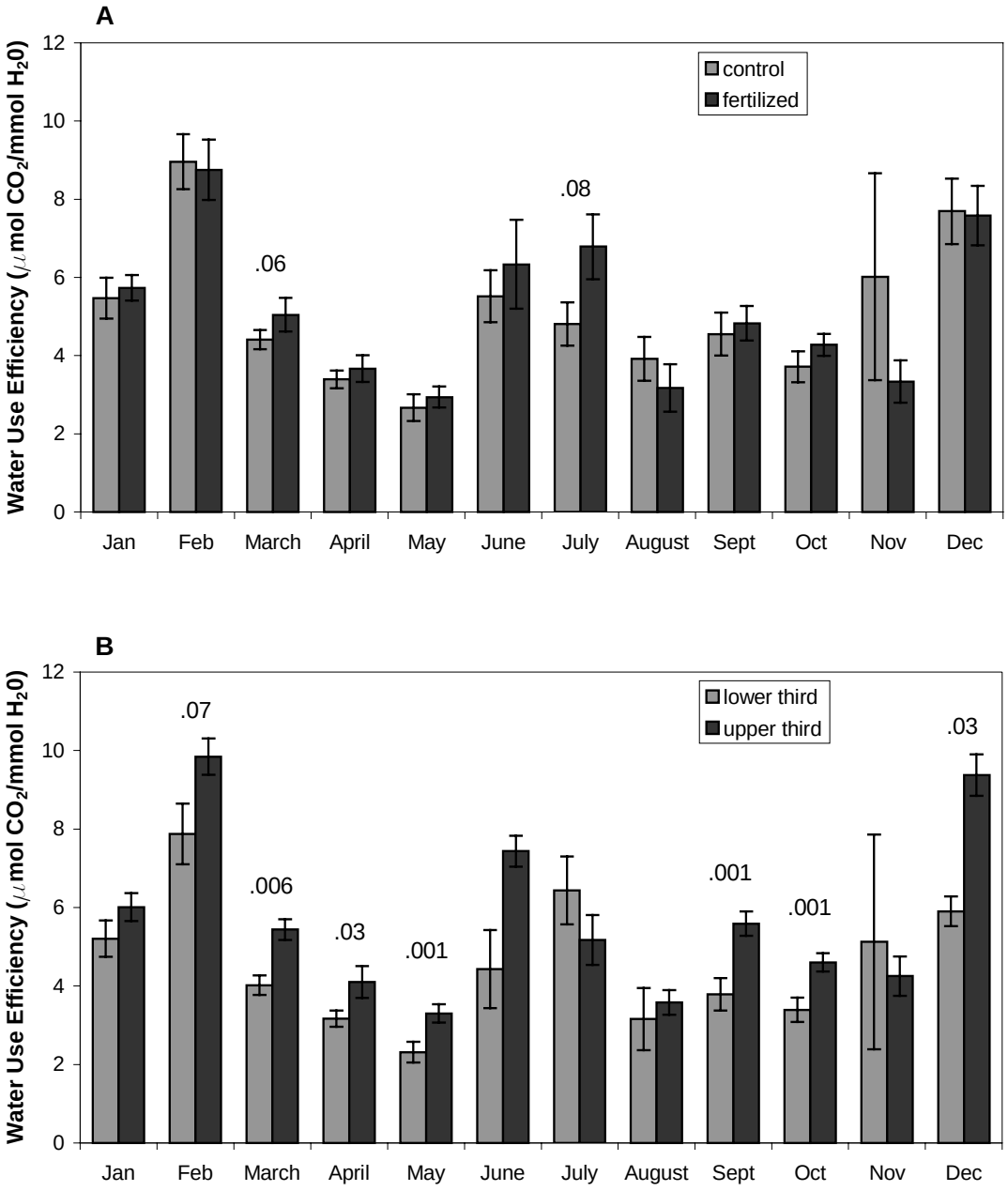

Figure 5: Mean monthly water use efficiencies in control and fertilized stands (A) and upper third and lower third of crowns (B). Bars indicate the standard error. Numbers above bars are p-values indicating significance levels between fertilization treatments or crown positions within months. P-values are only given when $p<0.1$. Each value is an average (n=16) from three measurement periods throughout a single day, except for November and December when morning measurements were not used in the analysis because of rain.

($p<0.1$). WUE was highest in both fertilized and control plots during February, reaching a value of approximately 9 μmol CO_2/ mmol H_2O. Upper crown foliage had higher mean WUE than lower crown foliage for all months except November, and significant differences occurred during February, March, April, May, September, October, and November ($p<0.1$).

Diurnal Trends in Gas Exchange

Photosynthesis

Photosynthesis was compared between fertilization treatments and crown positions for intensive measurement periods in January and July. Few significant differences in photosynthesis rates exist between control and fertilized stands during both January (Figure 6A,B) and July (Figure 7A,B). However, in July mean photosynthesis was generally greater in control stands while the opposite trend was apparent in January. Maximum mean net photosynthesis rates were fairly similar for both January and July intensive measurement periods, reaching average rates of approximately 3.5 μmol m^{-2} s^{-1} for the 10:30 a.m. measurement period in January and 4 μmol m^{-2} s^{-1} at 9:00 a.m. in July.

Photosynthesis differed significantly between the upper third of crowns and the lower third of crowns during all measurement periods both for the January (Figure 8A,B) and July (Figure 9A,B) intensive measurement periods ($p<0.01$). Maximum mean photosynthesis rates in the upper third of crowns reach approximately 4 μmol m^{-2} s^{-1} close to noon during January and almost 6 μmol m^{-2} s^{-1} around 9 a.m. in July. Lower crown mean photosynthesis reached a maximum rate of about 2.5 μmol m^{-2} s^{-1} in January for the 10:30 a.m. measurement sequence and approximately 2 μmol m^{-2} s^{-1} for the 9 a.m. measurement period in July. Lowest photosynthesis rates occurred close to dawn and dusk in both January and July which coincided with relatively low PAR values for the days sampled (Figures 10, 11). July photosynthesis appeared to peak sharply in the morning (around 9 a.m.) on both measurement days, and decrease notably in the late morning or early afternoon by as much as 40%.

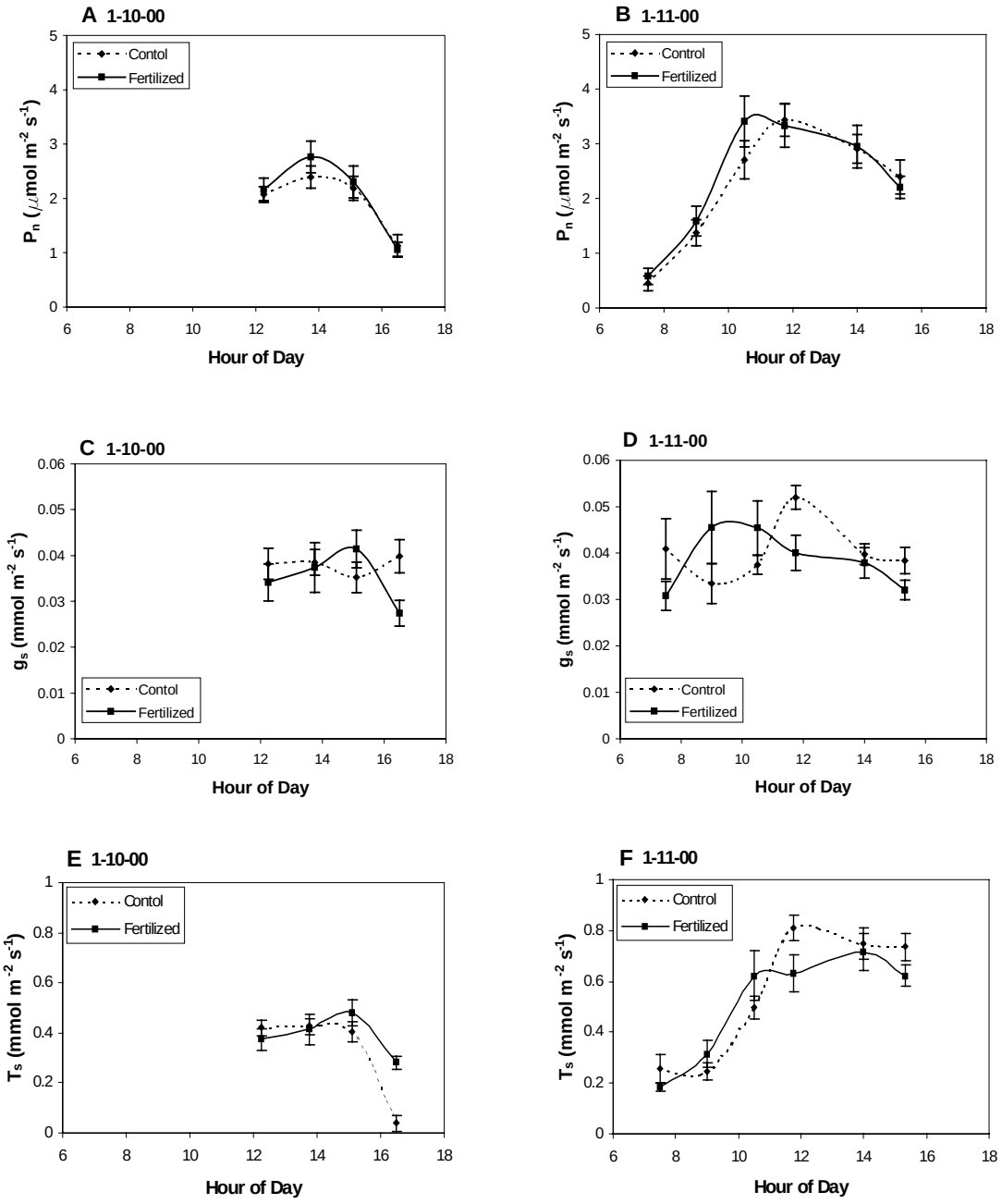

Figure 6: Net photosynthesis (A, B), conductance (C, D), and transpiration (E, F) in control and fertilized stands throughout the days of January 10 and 11, 2000. Each data point represents an average of 8 samples taken within an hour of the time indicated. Stars indicate a significant difference in gas exchange between crown positions ($p<0.1$). Bars indicate the standard error.

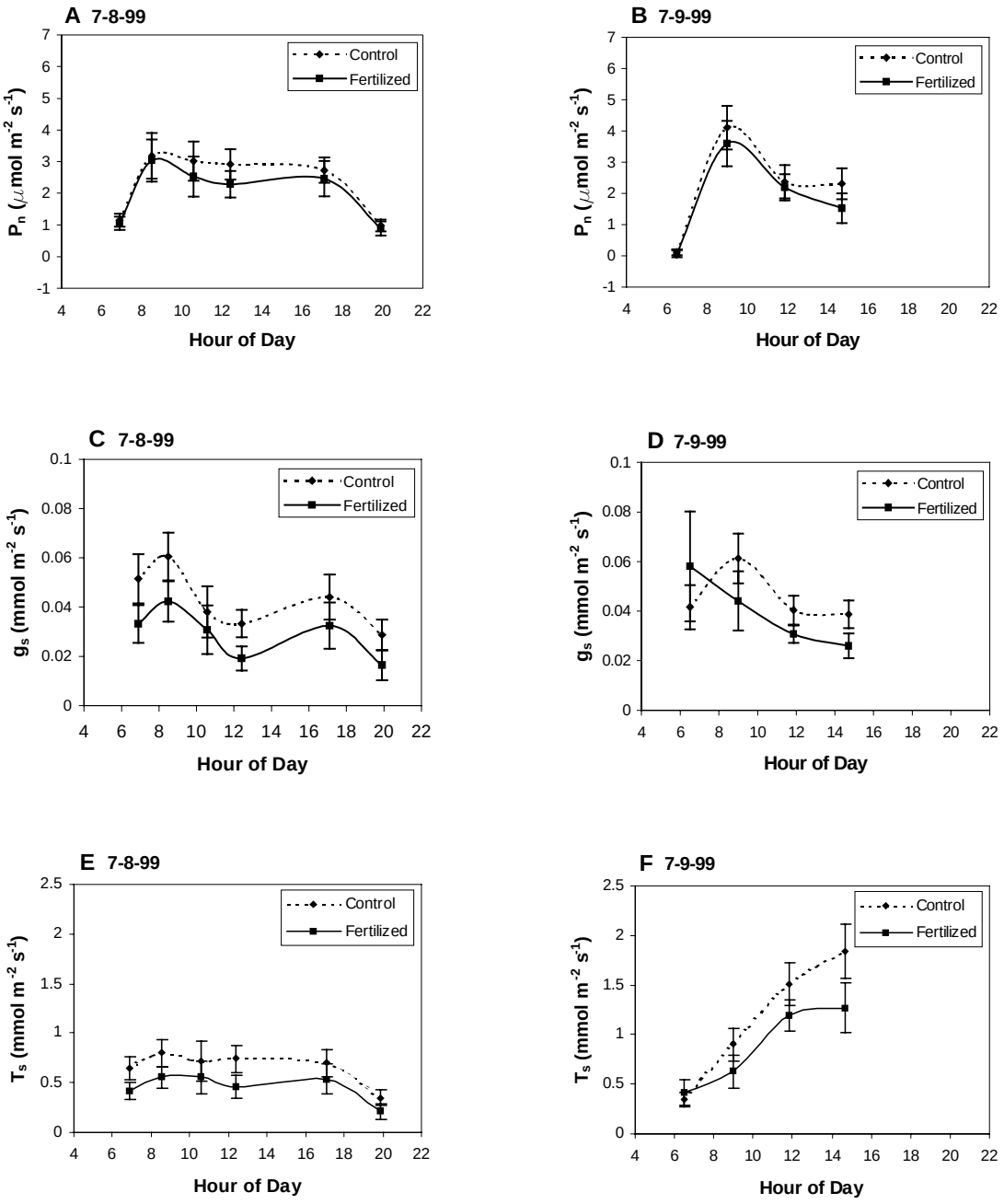

Figure 7: Net photosynthesis (A, B), conductance (C, D), and transpiration (E, F) in control and fertilized stands throughout the days of July 8 and 9, 2000. Each data point represents an average of 8 samples taken within an hour of the time indicated. Stars indicate a significant difference in gas exchange between crown positions ($p<0.1$). Bars indicate the standard error.

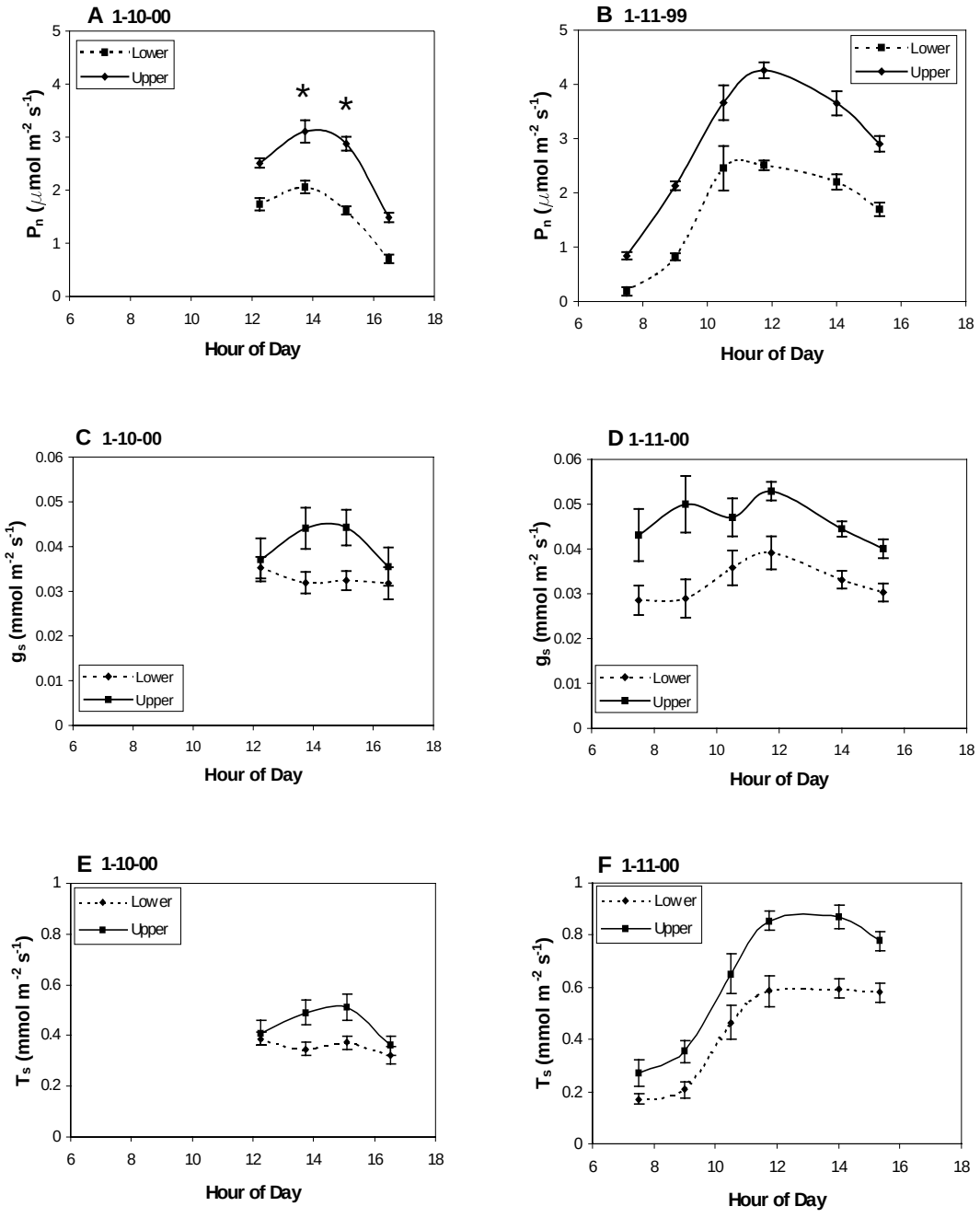

Figure 8: Net photosynthesis (A, B), conductance (C, D), and transpiration (E, F) in the lower and upper thirds of crowns throughout the days of January 10 and 11, 2000. Each data point represents an average of 8 samples taken within an hour of the time indicated. Stars indicate a significant difference in gas exchange between crown positions ($p<0.1$). Bars indicate the standard error.

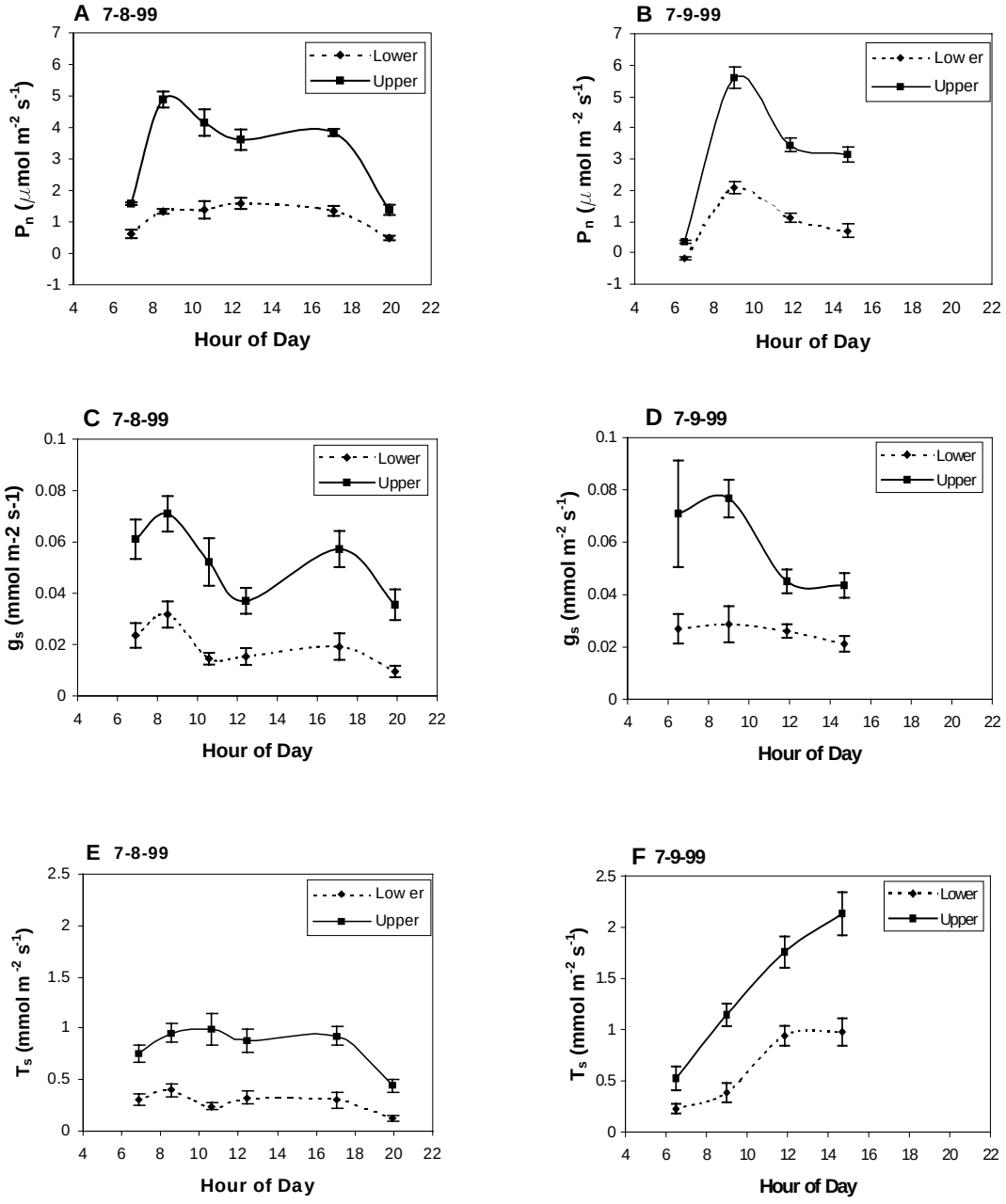

Figure 9: Net photosynthesis (A, B), conductance (C, D), and transpiration (E, F) in the lower and upper thirds of crowns throughout the days of July 8 and 9, 1999. Each data point represents an average of 8 samples taken within an hour of the time indicated. Stars indicate a significant difference in gas exchange between crown positions ($p<0.1$). Bars indicate the standard error.

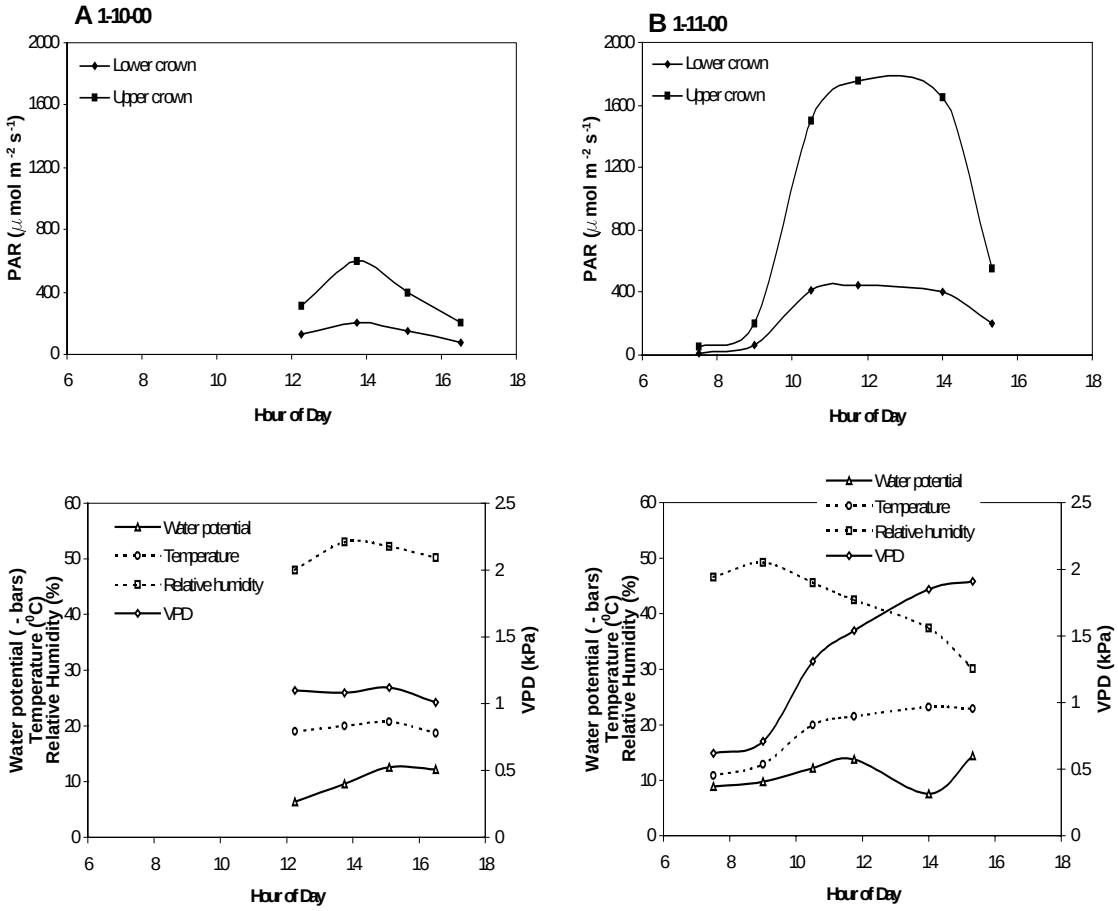

Figure 10: Mean upper and lower crown PAR, water potential, temperature, relative humidity, and VPD for each measurement period during the January intensive data collection. Environmental data from both day one (A), and day two (B) are given.

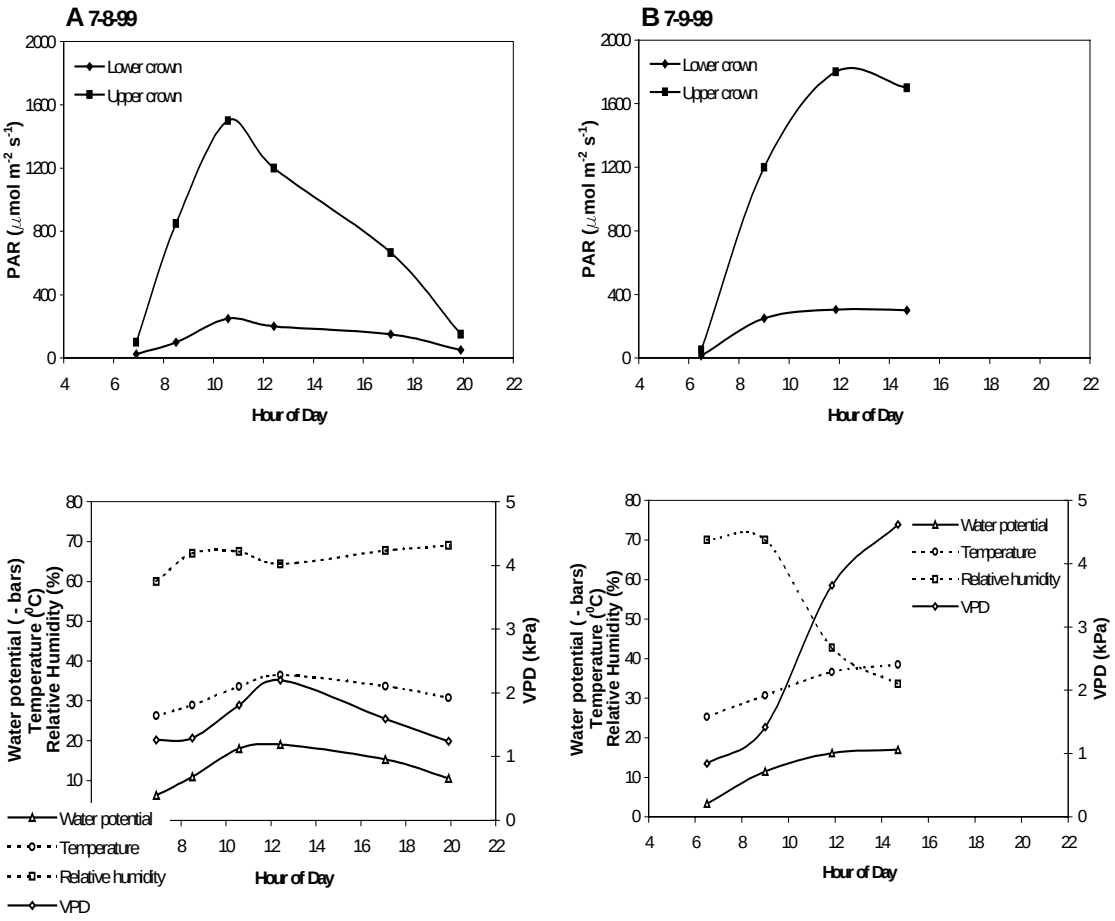

Figure 11: Mean upper and lower crown PAR, water potential, temperature, relative humidity, and VPD for each measurement period during the July intensive data collection. Environmental data from both day one (A), and day two (B) are given.

Conductance

Mean conductance values were also examined for differences between fertilization treatments and crown positions during the January and July intensive measurement periods. As with photosynthesis, few significant differences in conductance rates were apparent between foliage from control and fertilized stands during January (Figure 6 C,D) and July (Figure 7 C,D) measurement periods. No trends with respect to mean rates were obvious during January, but mean conductance was generally higher in control stands during July. When significant differences between treatments existed, control stands had higher rates. This was true for both months. Maximum mean conductance rates were achieved in the control stands for both months. In January conductance peaked at 0.05 mmol m^{-2} s^{-1} around noon and mean rates reached 0.06 mmol m^{-2} s^{-1} during the 9 a.m. measurement period in July.

Trends in conductance between the upper and lower thirds of crowns were much more clearly defined in both January (Figure 8C,D) and July (Figure 9C,D). The upper third of crowns had significantly greater mean conductance rates during a majority of measurement periods in January and during all measurement times in July ($p<0.01$). Conductance rates fluctuated throughout the day less during January than July. In January, conductance in both canopy positions stayed within a range slightly greater than 0.01 mmol m^{-2} s^{-1}. In July, mean lower crown conductance changed approximately 0.02 mmol m^{-2} s^{-1} in the first day, but varied much less on the second day. The upper crown consistently fluctuated within a range of about 0.04 mmol m^{-2} s^{-1} throughout the day on both days. Conductance roughly paralleled photosynthesis in the upper crown on both days in July. Maximum conductance values corresponded to maximum photosynthesis rates for both months. Maximum mean conductance in the lower crown was similar for both January and July. In January, the lower crown foliage reached a mean conductance of almost 0.04 mmol m^{-2} s^{-1} while July conductance was slightly lower, reaching about 0.03 mmol m^{-2} s^{-1}. In the upper third of crowns, January had a maximum mean conductance rate of over 0.05 mmol m^{-2} s^{-1} over the two days while July reached almost 0.08 mmol m^{-2} s^{-1}. Rates between crown positions differed by a greater margin in July.

Transpiration

An examination of differences in transpiration rates between fertilization treatments and crown positions revealed similar trends as those outlined for photosynthesis and conductance. In January, transpiration rates between fertilization treatments only differed for two measurement periods over the two days of measurements ($p<0.01$) (Figure 6E,F) and July comparisons reveal no significant differences between treatments (Figure 7E,F). Transpiration rates varied throughout the days during both months. Transpiration rates varied within a day as much as 0.6 mmol m^{-2} s^{-1} in the control stands during January and up to 1.5 mmol m^{-2} s^{-1} during a single day in July. Mean maximum transpiration differed greatly between the two months, reaching 0.8 mmol m^{-2} s^{-1} in January and almost 2 mmol m^{-2} s^{-1} in July.

Transpiration rates were significantly higher in the upper third of the crowns compared to the lower third of the crowns during a majority of measurement periods in January ($p<0.01$) (Figure 8E,F) and for all measurement periods in July ($p<0.01$) (Figure 9E,F). Rates varied throughout the day by as much as 0.4 mmol m^{-2} s^{-1} in the lower crown during January and in the upper crown by 0.6 mmol m^{-2} s^{-1}. In July, rates varied much more for the same crown position. The lower crown position fluctuated by as much as 0.75 mmol m^{-2} s^{-1} throughout the day and by over 1.5 mmol m^{-2} s^{-1} in the upper third of the crown on the same day. As with conductance, the marginal difference in rates between the upper and lower thirds of crowns was greater in July.

While transpiration roughly mirrored photosynthesis in January, July results varied. On the first measurement day in July, transpiration rates remained relatively low throughout the day in both fertilization treatments and crown positions. Transpiration rose steadily throughout the second day, reaching a maximum during the last measurement period (around 3 p.m.). This rise in transpiration corresponded to a drop in photosynthesis. Conductance more closely paralleled photosynthesis during the July intensive measurements, while transpiration more closely matched changes in photosynthesis throughout the second day in January.

Modeling Gas Exchange

Overview

Common models for all fertilization treatment and crown position combinations were developed (1) to explain the relationship between gas exchange and the environment, (2) to compare gas exchange response surfaces among fertilization treatment and crown position combinations, and (3) to develop empirical quantitative relationships that will be utilized in process modeling efforts. Common models were chosen based on stepwise model comparisons among all fertilization treatment and crown position combinations. Criteria for common model selection included common significant variables, similar overall model R^2, and a simple overall model (five variables or less). Initial intentions were to model photosynthesis, conductance, and transpiration. However, the stepwise procedure in SAS revealed that no sufficient common model for conductance existed among the four fertilization and crown position combinations (Table 1). Results from the stepwise procedure indicated that several variables explained only a fractional amount of the variation in conductance values, and these variables were not consistently significant in all models. R^2 values in preliminary common models ranged from only 0.05 to a maximum of 0.25. This suggests that variation in conductance is not explained sufficiently by the ambient environment and conductance is relatively unresponsive to the environment in loblolly pine. Also, measurement error (due to machine limitations) may be relatively high. Therefore, only photosynthesis and transpiration models are presented in the following sections.

Predicted net photosynthesis and transpiration is shown along with actual model parameter estimates in the following sections. The independent variable chosen in figures showing predicted net photosynthesis is PAR, since light intensity explains a majority of the variation in photosynthesis. Default values for other model variables were chosen that occur within the typical, reasonable range of values in all fertilization treatment and crown position combination. Mean environmental parameters for each month are listed in Table 2. The default value for VPD in all photosynthesis prediction models is 2.0 kPa and relative humidity was assigned a value of 50% (for models developed from intensive measurement periods only). The independent variable in

Table 1: Summary of SAS stepwise procedure in which conductance is the dependent variable for all fertilization and crown position combinations. Only variables having partial R^2 values greater than 0.02 are given. For all variables, p-values<0.15.

Parameter	Partial Model R^2
Control Treatment, lower crown position	n=398
VPD x Relative humidity	0.094
Log(VPD)	0.075
Log(air temperature)	0.055
√(Air temperature)	0.03
VPD	0.03
(Relative humidity)^2	0.02
Ln(PAR)	0.02
Control Treatment, upper crown position	n=404
VPD^2	0.03
Log(air temperature)	0.08
PAR x relative humidity	0.02
VPD x relative humidity	0.01
Fertilized Treatment, lower crown position	n=393
VPD x relative humidity	0.18
PAR x relative humidity	0.06
PAR	0.02
Log(air temperature)	0.02
Fertilized Treatment, upper crown position	n=405
Log(VPD)	0.04
PAR x temperature	0.03
Log(air temperature)	0.02
(Relative humidity)^2	0.03
(Air temperature)^2	0.03
√(Relative humidity)	0.06
VPD^2	0.05
VPD^3	0.11

Table 2: Mean PAR in the lower and upper third of crowns, mean VPD, mean air temperature, mean relative humidity, and mean water potential for each month during measurements. Each value represents an average from the three measurement periods (morning, early afternoon, late afternoon) from a single day. Mean PAR in the lower and upper third of crowns is an average of 8 samples. All other environmental variables represent an average from 16 samples.

Month	Mean lower crown PAR (μmol m^{-2} s^{-1})	Mean upper crown PAR (μmol m^{-2} s^{-1})	Mean VPD (kPa)	Mean Air Temperature (°C)	Mean Relative Humidity (%)	Mean Water Potential (Bars)
January	310	1154	1.2	18.1	45.8	11.9
February	450	1200	0.88	12.8	42.4	10.0
March	374	1093	1.6	19.2	28.2	13.7
April	348	858	2.1	28.6	47.6	12.7
May	471	1233	2.7	30.7	42.8	14.7
June	74	283	1.0	23.7	62.7	7.6
July	183	1183	1.8	33.0	66.4	16.0
August	258	1066	3.2	34.3	47.9	16.6
September	251	1383	1.4	21.2	48.0	11.0
October	244	1166	1.3	17.9	37.5	10.9
November	148	508	0.86	23.9	70.0	7.4
December	66	151	0.61	12.3	53.7	7.1

figures showing predicted transpiration is VPD since this variable consistently explains a majority of the variation in transpiration. The default value for PAR was assigned 500 μmol m^{-2} s^{-1} and relative humidity was given a value of 50% in all transpiration prediction models.

Models for Photosynthesis and Transpiration Based on Data from the Entire Year

Data from the entire year were used to develop empirical models for photosynthesis and transpiration. Actual gas exchange data and predicted values plotted against environmental parameters are presented in Appendix A and B. The common model for photosynthesis includes the following parameters: PAR, Ln(PAR), and VPD (Table 3). Statistical comparisons of parameter estimates reveal that significant differences exist among parameter estimates for the four fertilization treatment and crown position combinations, indicating that individual models are necessary. Statistical tests indicate that the y-intercept estimate is lower for the upper third of crowns in both fertilized and control stands ($p<0.01$), suggesting that higher respiration rates occur in the upper third of the crowns. Also, Ln(PAR) parameter estimates are significantly greater for the upper third of the crowns in comparison to the lower third of the crowns in both fertilized and control stands ($p<0.01$), indicating a greater responsiveness to light in the upper third of the canopy. Finally, slope comparisons show that VPD parameter estimates are significantly more negative in fertilized stands compared to the control stands for both crown positions ($p<0.05$), which suggests that foliage from fertilized stands is more sensitive to VPD (or a greater reduction in photosynthesis occurs with increasing VPD).

Figure 12A shows the predicted relationship between Photosynthesis and PAR. Upper crown foliage from both fertilization treatments has higher predicted photosynthesis rates than lower crown foliage at the same light level, suggesting that upper crown foliage has a greater quantum yield at higher light levels. Although predicted P_{max} (maximum photosynthesis) in the upper crown reaches about 4 μmol m^{-2} s^{-1} in both control and fertilized stands, the control stands have higher predicted rates except at the highest PAR levels (>1500 μmol m^{-2} s^{-1}). A_{sat} (light-

Table 3: Photosynthesis parameter estimates for common models of all fertilization treatment and crown position combinations based on data from the entire year, corresponding p-values, and total model R^2 values. Stars next to parameters indicate estimates differ significantly between at least two treatment combinations ($P<0.05$).

Parameter	Estimate	P-value	Model R^2
Control treatment, lower crown position		n=416	
Intercept*$^{\blacklozenge}$	-2.245	0.0001	0.58
PAR*	2.388×10^{-4}	0.04	
Ln(PAR)*$^{\bullet}$	0.8471	0.0001	
VPD*$^{\Delta}$	-0.3048	0.0001	
Control treatment, upper crown position		n=415	
Intercept*$^{\blacklozenge}$	-5.605	0.0001	0.56
PAR*	-7.884×10^{-4}	0.0002	
Ln(PAR)*$^{\bullet}$	1.540	0.0001	
VPD*$^{\Delta}$	-0.1927	0.0002	
Fertilized treatment, lower crown position		n=415	
Intercept*$^{\blacklozenge}$	-1.469	0.0001	0.58
PAR*	0.001038	0.0007	
Ln(PAR)*$^{\bullet}$	0.6771	0.0001	
VPD*$^{\Delta}$	-0.4486	0.0001	
Fertilized treatment, upper crown position		n=414	
Intercept*$^{\blacklozenge}$	-4.571	0.0001	0.56
PAR*	-2.482×10^{-4}	0.23	
Ln(PAR)*$^{\bullet}$	1.368	0.0001	
VPD*$^{\Delta}$	-0.5134	0.0001	

*$^{\blacklozenge}$ All slope test comparisons show that the intercept estimate is significantly lower for the upper third of the crown compared to the lower third of the crown in both fertilized and control stands ($p<0.01$), indicating higher respiration rates occur in the upper third of the crown.

*$^{\bullet}$ All slope test comparisons show that Ln(PAR) parameter estimates are significantly greater for the upper third of the crown in comparison to the lower third of the crown in both fertilized and control stands ($P<0.01$), indicating a greater responsiveness to increasing light intensity in the upper third of the canopy.

*$^{\Delta}$ All slope comparisons show that VPD parameter estimates are significantly more negative in the fertilized stands compared to the control stands for both crown positions ($p<0.05$), indicating a greater sensitivity to VPD in the fertilized stands (i.e. a greater reduction in Pn with increasing VPD).

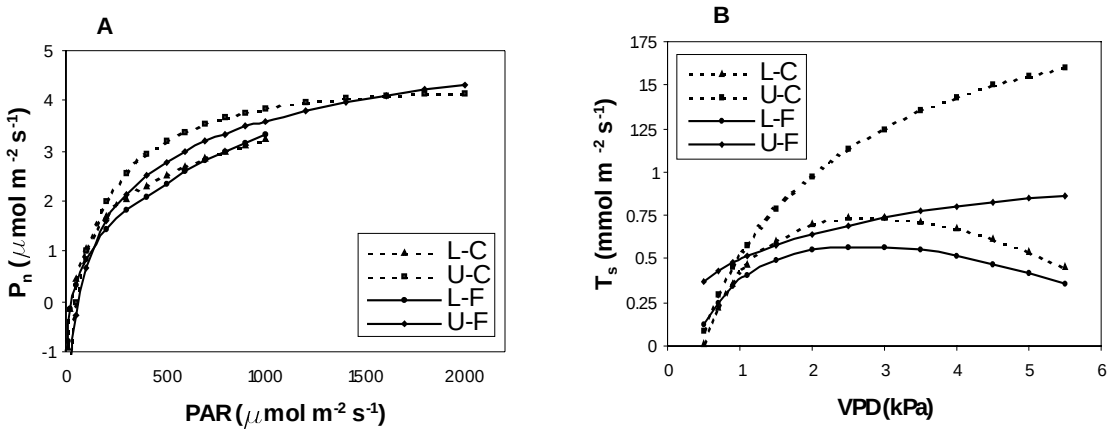

Figure 12: Predicted net photosynthesis in relation to PAR (A) and transpiration in relation to VPD (B) based on data from the entire year. L = lower third of crown, U = upper third of crown, C = control stands, F = fertilized stands.

saturated photosynthesis) is about 1250 μmol m^{-2} s^{-1} in the upper crown of control plots, but photosynthesis does not clearly become saturated in the upper crown of fertilized plots.

The common model among all fertilization treatments and crown position combinations for transpiration includes the following variables: Log(VPD), VPD^2, VPD x PAR, and VPD x Relative humidity (Table 4). Statistical comparisons show that the parameter estimates for Log(VPD), VPD^2, and VPD x PAR of at least two fertilization treatment and crown position combinations differ significantly, demonstrating the need for separate models ($P<0.1$).

The predicted relationship between transpiration and VPD is shown in Figure 12B. Upper crown foliage from fertilized stands appears to be least responsive to VPD. Transpiration is generally predicted to be lower in fertilized stands for both crown positions. Lower crown foliage from fertilized stands is predicted to have lower rates than those predicted for lower crown foliage from control stands. Both lower crown positions are predicted to reach a maximum transpiration rate and threshold when VPD reaches approximately 2.5 kPa. Rates in the lower crown are predicted to reach a maximum of 0.5 mmol m^{-2} s^{-1} in fertilized stands and slightly under 0.75 mmol m^{-2} s^{-1} in control stands. Likewise, foliage from the upper crown of fertilized stands has much lower predicted transpiration rates in comparison to control stands, especially at higher VPD values. However, unlike the lower crown foliage, transpiration rates never reach a threshold in which rates decline after a given VPD. Maximum values in the upper crown are predicted to occur at the highest VPD values. In control stands maximum predicted transpiration is over 1.5 mmol m^{-2} s^{-1} at 5 kPa, but only slightly greater than 0.75 mmol m^{-2} s^{-1} in fertilized stands for the same VPD.

Photosynthesis models

Growing and Non-Growing Seasons

Models for both the growing and the non-growing seasons were developed to examine seasonal differences in how gas exchange responds to the environment. Within the growing season and non-growing season, predicted net photosynthesis varied

Table 4: Transpiration parameter estimates for common models of all fertilization treatment and crown position combinations based on data for the entire year, corresponding p-values, and total model R^2 values. Stars next to parameters indicate estimates differ significantly between at least two treatment combinations ($P<0.1$).

Parameter	Estimate	P-value	Model R^2
Control treatment, lower crown position		n=398	
Intercept*	0.6324	0.0001	0.48
Log(VPD)*	1.714	0.0001	
VPD^2*	-0.01095	0.023	
VPD x PAR*	7.335×10^{-5}	0.031	
VPD x Relative humidity	-0.004803	0.0001	
Control treatment, upper crown position		n=404	
Intercept*	0.4217	0.0001	0.62
Log(VPD) *	1.270	0.0001	
VPD^2*	-0.01252	0.088	
VPD x PAR*	8.063×10^{-5}	0.0003	
VPD x Relative humidity	0.001428	0.13	
Fertilized treatment, lower crown position		n=393	
Intercept*	0.5266	0.0001	0.38
Log(VPD)*	1.113	0.0001	
VPD^2*	-0.007493	0.063	
VPD x PAR*	1.101×10^{-4}	0.0014	
VPD x Relative humidity	-0.003909	0.0001	
Fertilized treatment, upper crown position		n=405	
Intercept*	0.4119	0.0001	0.44
Log(VPD) *	0.2720	0.16	
VPD^2*	-0.008647	0.26	
VPD x PAR*	1.257×10^{-4}	0.0001	
VPD x Relative humidity	5.838×10^{-4}	0.54	

depending on the fertilization treatment and crown position combination (Figure 13). Statistical comparison of parameter estimates among fertilization treatment and crown position combinations show that significant differences exist among variable estimates ($p<0.1$). Therefore, models were developed for all fertilization treatment and crown position combinations.

Within the growing season, upper crown foliage in control stands has higher predicted photosynthesis rates than fertilized plots for a given light level. P_{max} values appear to converge at the highest light intensity, reaching a maximum predicted rate of about 4.5 μmol m^{-2} s^{-1} when the PAR level is 2000 μmol m^{-2} s^{-1}. A_{sat} in the upper crown occurs at similar PAR values for both fertilization treatments, saturating above 1500 μmol m^{-2} s^{-1}. Lower crown foliage has lower predicted photosynthesis than upper crown foliage for both fertilization treatments, except for at the lowest light levels. Lower crown foliage during the growing season has a higher initial predicted quantum yield. Control plots generally have slightly higher predicted photosynthesis in the lower crown than fertilized plots for a defined light level. Respiration rates (based on intercepts) are predicted to be higher in upper crown foliage for both fertilization treatments.

During the non-growing season, predicted photosynthesis is also generally higher in the control stands compared to the fertilized stands, except at the highest PAR levels. In the upper crown, photosynthesis is expected to be greater in control stands until PAR levels become greater than 1300 μmol m^{-2} s^{-1}. Predicted P_{max} is approximately 4 μmol m^{-2} s^{-1} in the upper crown of control stands while photosynthesis in the upper crown of fertilized stands is not saturated within the range of light intensities presented. Rates are predicted to reach roughly 4.5 μmol m^{-2} s^{-1} at a light intensity of 2000 μmol m^{-2} s^{-1} in upper foliage of fertilized stands. A_{max} for upper crown foliage in control stands is predicted to occur when PAR reaches slightly over 1000 μmol m^{-2} s^{-1}. Predicted intercept values are lower in the upper crown for both fertilization treatments compared to the lower crown, again suggesting that respiration is greater in the upper crown.

Actual parameter estimates and the statistical comparison of variable estimates between the growing and non-growing seasons confirm that the environment influences photosynthesis differently depending on the season (Table 5). At least two (of four) parameter estimates for the growing and non-growing seasons differed statistically for all

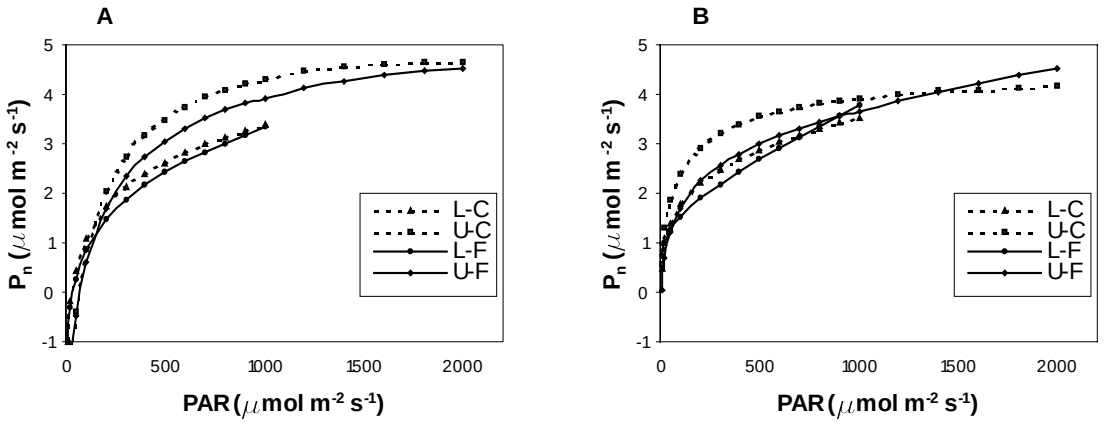

Figure 13: Predicted net photosynthesis in relation to PAR for the growing season (A) and the non-growing season (B). L = lower third of crown, U = upper third of crown, C = control stands, F = fertilized stands.

Table 5: Photosynthesis prediction model parameter estimates, corresponding p-values, and model R^2 for the growing season (April – October) and non-growing (November – March) season for all fertilization treatment and crown position combinations. Stars next to parameters indicate estimates differ significantly between growing and non-growing season ($P<0.1$).

Control treatment, lower crown position

Parameter	Estimate	P-value	Model R^2
Growing season		n=240	
Intercept	-2.295	0.0001	0.58
PAR	2.816×10^{-4}	0.4159	
Ln(PAR)*	0.8949	0.0001	
VPD*	-0.3924	0.0001	
Non-growing season		n=176	
Intercept	-1.717	0.0001	0.64
PAR	5.497×10^{-4}	0.2086	
Ln(PAR)*	0.5466	0.0001	
VPD*	0.4673	0.0004	

Control treatment, upper crown position

Parameter	Estimate	P-value	Model R^2
Growing season		n=239	
Intercept*	-6.689	0.0001	0.64
PAR*	-9.561×10^{-4}	0.0003	
Ln(PAR)*	1.876	0.0001	
VPD*	-0.4976	0.0001	
Non-growing season		n=176	
Intercept*	-2.870	0.0005	0.63
PAR*	-3.303×10^{-4}	0.20	
Ln(PAR)*	0.7980	0.0001	
VPD*	0.8098	0.0001	

Table 5. continued

Fertilized treatment, lower crown position

Parameter	Estimate	P-value	Model R^2
Growing season		n=240	
Intercept*	-1.802	0.0001	0.59
PAR*	7.237×10^{-4}	0.12	
Ln(PAR)*	0.7912	0.0001	
VPD*	-0.5238	0.0001	
Non-growing season		n=175	
Intercept*	-0.4707	0.17	0.63
PAR*	0.001816	0.0001	
Ln(PAR)*	0.2684	0.004	
VPD*	0.2911	0.02	

Fertilized treatment, upper crown position

Parameter	Estimate	P-value	Model R^2
Growing season		n=239	
Intercept*	-5.542	0.0001	0.60
PAR*	5.477×10^{-4}	0.042	
Ln(PAR)*	1.653	0.0001	
VPD*	-0.6983	0.0001	
Non-growing season		n=175	
Intercept*	-2.048	0.025	0.62
PAR*	3.670×10^{-4}	0.20	
Ln(PAR)*	0.7066	0.0003	
VPD*	0.2356	0.20	

fertilization treatment and crown position combinations ($p<0.01$), indicating that separate models for the growing and non-growing seasons are necessary. Predicted net photosynthesis in response to PAR was also compared graphically to illustrate differences between the growing and non-growing seasons for each fertilization treatment and crown position combination (Figure 14). Interestingly, predicted photosynthesis in the lower crown is greater during the non-growing season for both control and fertilized stands. Predicted responses in the lower crown are practically parallel for both the growing and non-growing seasons.

The predicted response to PAR in the upper crown differs significantly between the growing and non-growing seasons. At low light levels (PAR<500 μmol m^{-2} s^{-1}) in both control and fertilized stands, foliage from the non-growing season is more responsive to light (indicating a greater initial quantum yield). At PAR levels greater than 500 μmol m^{-2} s^{-1}, foliage from the growing season has a higher predicted photosynthesis rate. The predicted P_{max} value in the control stands is greater for the growing season, reaching a maximum of over 4.5 μmol m^{-2} s^{-1} for the growing season and approximately 4.0 μmol m^{-2} s^{-1} during the non-growing season. Predicted A_{sat} in the control stands also differs. During the growing season, A_{sat} is reached at a PAR of about 1500 μmol m^{-2} s^{-1} while the non-growing season model predicts light saturation at closer to 1000 μmol m^{-2} s^{-1}. In fertilized stands maximum photosynthesis was not achieved within the range of PAR values presented, but predicted values for both the growing and non-growing season converged at a PAR of 2000 μmol m^{-2} s^{-1}.

Predicted intercepts are lower for all fertilization treatment and crown position combinations during the growing season, indicating that higher respiration rates occur during the growing season.

January and July Intensive Measurement Periods

Photosynthesis prediction models were developed for the January and July intensive measurement periods to examine potential differences between models for the entire growing and non-growing seasons and models based on data collected intensively throughout two consecutive days within the two seasons. Development of models for both entire seasons and from only two consecutive days of data allowed for the

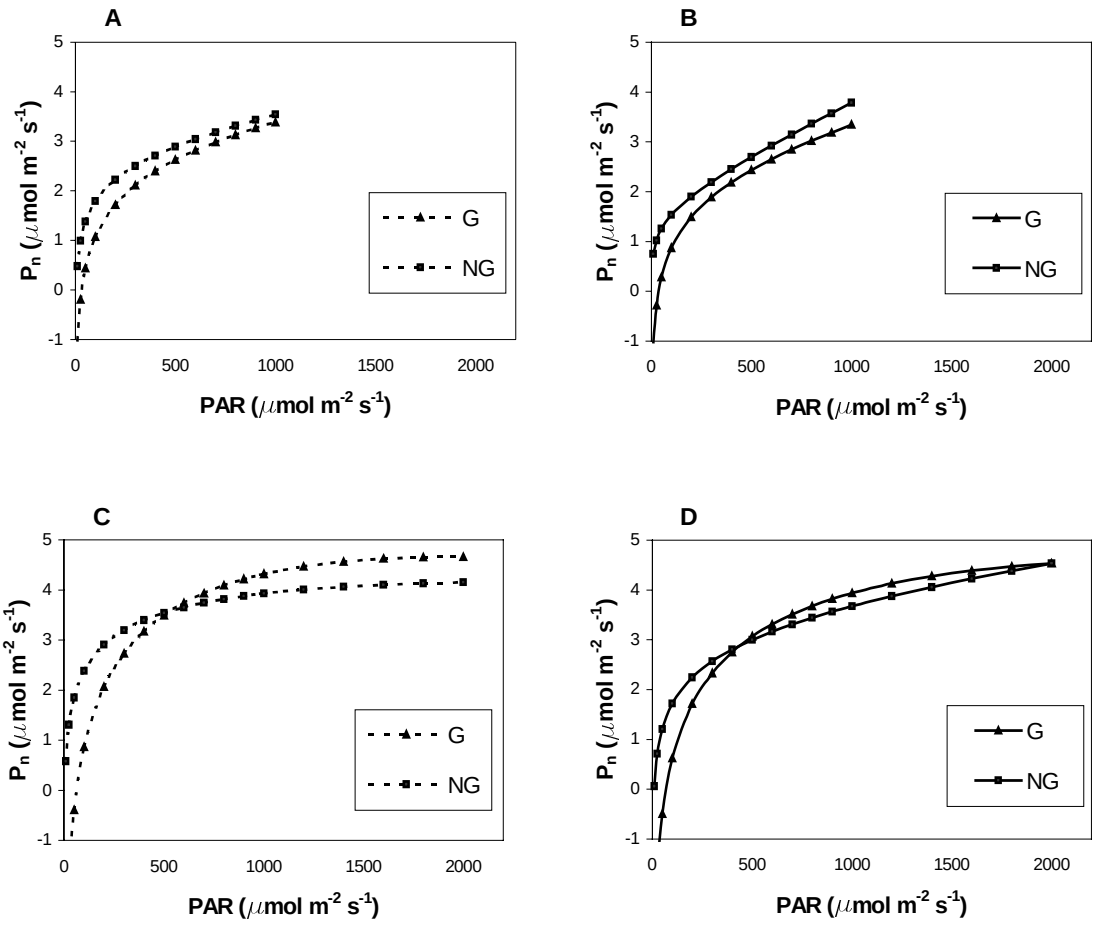

Figure 14: Predicted net photosynthesis in response to PAR for the growing season and non-growing season for each fertilization treatment and crown position combination. Lower third of crown and control stands (A), lower third of crown and fertilized stands (B), upper third of crown and control stands (C), upper third of crown and fertilized stands (D). G = growing season (April – October), NG = non-growing season (November – March).

examination of both seasonal and daily variation in gas exchange. Also, prediction models developed from the intensive measurement periods were compared statistically.

Common model parameters (PAR, Ln(PAR), and VPD) were compared statistically between seasonal models and intensive models for all fertilization treatment and crown position combinations. R^2 values were generally higher for models developed from January intensive data compared to the entire non-growing season, demonstrating that more unexplained variation occurred over the non-growing season compared to two consecutive measurement days. This suggests that seasonal acclimation may occur, resulting in monthly variation in how the environment influences gas exchange.

One or more parameter estimates in all prediction models developed for fertilization treatment and crown position combinations from the January intensive measurement period and non-growing season statistically differ ($p<0.1$). Predicted intercepts in fertilized stands are significantly lower for the January intensive measurement period compared to the entire non-growing season. The slope estimate for the Ln(PAR) parameter is higher for fertilized stands in the January intensive model, indicating that foliage from fertilized stands in January has a greater predicted responsiveness to light than the model from the entire growing season predicts. These differences between the January intensive and non-growing season models are apparent when predicted net photosynthesis is plotted against PAR (Figure 15). Statistical comparisons among model parameters developed for fertilization treatment and crown position combinations within January resulted in trends similar to those outlined for the photosynthesis models developed for the entire non-growing season. The predicted intercepts are significantly lower in the upper crown, and upper crown foliage has a greater predicted response to light. It should be noted that predicted net photosynthesis in the lower crown was extrapolated beyond the range of actual data for comparison purposes, resulting in the inaccurate predicted values presented in Figure 15.

July intensive and growing season prediction models for all fertilization treatment and crown position combinations did not significantly differ ($p<0.1$). Predicted net photosynthesis in relation to PAR for both the July and growing season models reveals similar trends (Figure 16). However, some predicted responses do appear to differ,

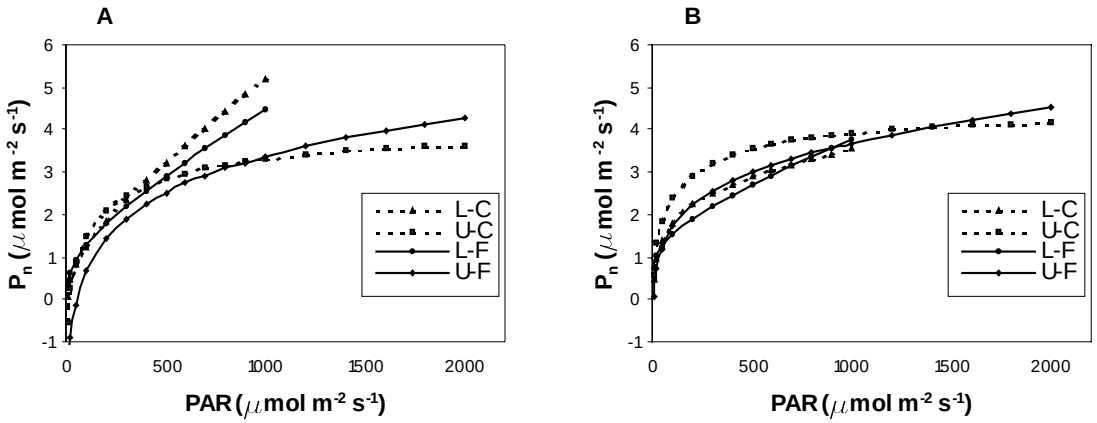

Figure 15: Predicted net photosynthesis in relation to PAR for the January intensive measurement period (A) and the entire non-growing season (B). L = lower third of crown, U = upper third of crown, C = control stands, F = fertilized stands.

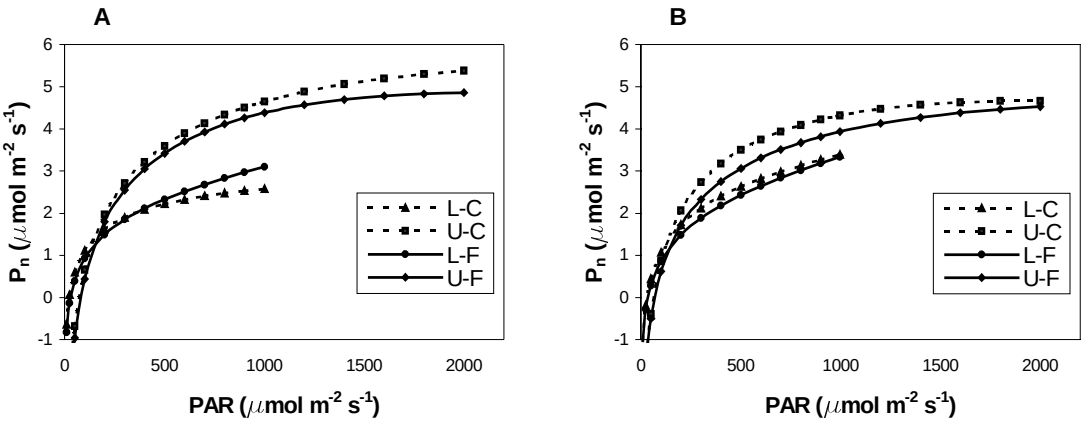

Figure 16: Predicted net photosynthesis in relation to PAR for the July intensive measurement period (A) and the entire growing season (B). L = lower third of crown, U = upper third of crown, C = control stands, F = fertilized stands.

although not statistically. Predicted lower crown photosynthesis is greater in fertilized stands above PAR values of 500 μmol m^{-2} s^{-1} in the July intensive model. Also, predicted P_{max} in the upper crown of control stands for the July intensive model reaches over 5 μmol m^{-2} s^{-1} at a PAR of 2000 μmol m^{-2} s^{-1} and light saturation does not appear to occur. The growing season model predicts that saturation occurs at a PAR of about 1500 μmol m^{-2} s^{-1} in the upper crown of control stands. As with the prediction model for the entire growing season, lower crown foliage has a higher predicted initial quantum yield than upper crown foliage.

Common models were developed for the January and July intensive periods that are slightly different from those previously used and include the following parameters: PAR x relative humidity, Ln(PAR), and VPD (Table 6). Parameter estimates statistically differ between models for all fertilization treatment and crown position combinations except January and July models for the upper crown of control stands ($p<0.1$). The VPD parameter estimate is only significant in one January intensive model (fertilized treatment, upper crown) suggesting that VPD did not significantly influence photosynthesis during the January measurement period. The varying predicted relationship of photosynthesis to PAR for the January and July intensive measurement periods closely resembles differences outlined between the non-growing and growing seasons (Figure 17). Predicted photosynthesis rates in the lower third of the crown are less in July than January. In the upper third of the crown, predicted P_{max} and A_{sat} are greatest during July. However, quantum yield in the upper crown of foliage is initially higher during January compared to July.

Transpiration Models

Growing and Non-Growing Seasons

Common transpiration prediction models developed for each fertilization treatment and crown position combination from the growing and non-growing seasons include the following variables: Log(VPD), VPD^2, VPD x PAR, and VPD x relative humidity (Table 7). Since VPD is the major environmental factor influencing transpiration, predicted transpiration was plotted against VPD to illustrate model variation for all treatment combinations within a season (Figure 18). Predicted

Table 6: Photosynthesis parameter estimates for common prediction models, corresponding p-values, and model R^2 values for the January and July intensive measurement periods for all fertilization treatment and crown position combinations. Stars next to parameters indicate estimates differ significantly between intensive measurement periods (P<0.1).

Control treatment, lower crown position

Parameter	Estimate	P-value	Model R^2
January		n=80	
Intercept	-0.8028	0.10	0.62
PAR x Relative humidity	6.907×10^{-5}	0.0074	
Ln(PAR)	0.3660	0.032	
VPD	-0.007255	0.98	
July		n=80	
Intercept	-1.673	0.035	0.45
PAR x Relative humidity	-7.083×10^{-6}	0.87	
Ln(PAR)	0.7744	0.0067	
VPD	-0.3741	0.0031	

Control treatment, upper crown position

Parameter	Estimate	P-value	Model R^2
January		n=80	
Intercept*	-2.931	0.0007	0.69
PAR x Relative humidity	-6.427×10^{-6}	0.32	
Ln(PAR)*	0.9114	0.0001	
VPD*	0.1374	0.61	
July		n=79	
Intercept*	-6.739	0.0001	0.79
PAR x Relative humidity	-1.276×10^{-5}	0.17	
Ln(PAR)*	1.982	0.0001	
VPD*	-0.8323	0.0001	

Table 6. continued

Fertilized treatment, lower crown position

Parameter	Estimate	P-value	Model R^2
January		n=80	
Intercept*	-0.4768	0.12	0.74
PAR x Relative humidity*	5.512×10^{-5}	0.0001	
Ln(PAR)*	0.3117	0.0026	
VPD*	0.02354	0.91	
July		n=80	
Intercept*	-1.680	0.015	0.49
PAR x Relative humidity*	1.017×10^{-5}	0.78	
Ln(PAR)*	0.7421	0.0028	
VPD*	0.4289	0.0001	

Fertilized treatment, upper crown position

Parameter	Estimate	P-value	Model R^2
January		n=80	
Intercept*	-3.306	0.0023	0.68
PAR x Relative humidity*	2.854×10^{-6}	0.72	
Ln(PAR)*	1.120	0.0001	
VPD	-0.6068	0.067	
July		n=79	
Intercept*	-7.021	0.0001	0.67
PAR x Relative humidity*	1.951×10^{-5}	0.077	
Ln(PAR)*	2.093	0.0001	
VPD	-1.038	0.0001	

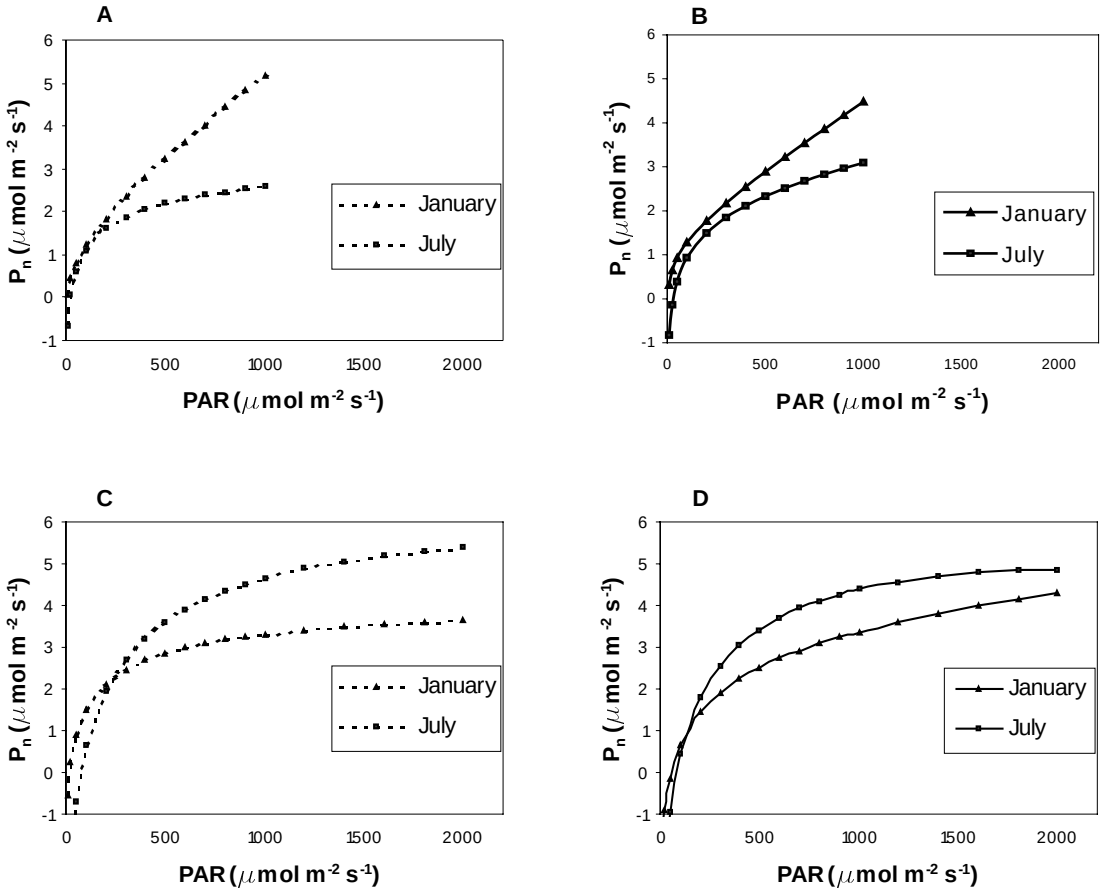

Figure 17: Predicted net photosynthesis in response to PAR for the July and January intensives for each fertilization treatment and crown position combination. Lower third of crown and control stands (A), lower third of crown and fertilized stands (B), upper third of crown and control stands (C), upper third of crown and fertilized stands (D).

Table 7: Transpiration parameter estimates for common models, corresponding p-values, and model R^2 for the growing season (April – October) and non-growing (November – March) season for all fertilization treatment and crown position combinations. Stars next to parameters indicate estimates differ significantly between growing and non-growing season (P<0.1).

Control treatment, lower crown position

Parameter	Estimate	P-value	Model R^2
Growing season		n=233	
Intercept*	0.8591	0.0001	0.47
Log(VPD)	2.866	0.0001	
VPD^2*	-0.02409	0.0004	
VPD x PAR*	3.083×10^{-5}	0.44	
VPD x Relative humidity*	-0.009047	0.0001	
Non-growing season		n=167	
Intercept*	0.05891	0.53	0.57
Log(VPD)	-0.3079	0.22	
VPD^2*	0.2462	0.0001	
VPD x PAR*	1.264×10^{-4}	0.10	
VPD x Relative humidity*	-2.906×10^{-4}	0.86	

Control treatment, upper crown position

Parameter	Estimate	P-value	Model R^2
Growing season		n=240	
Intercept*	0.6170	0.0001	0.53
Log(VPD)	1.598	0.0005	
VPD^2	-0.01867	0.067	
VPD x PAR*	1.016×10^{-4}	0.0017	
VPD x Relative humidity*	-0.001222	0.49	
Non-growing season		n=167	
Intercept*	0.08067	0.28	0.68
Log(VPD)	-0.5565	0.0031	
VPD^2	0.2969	0.0001	
VPD x PAR*	2.094×10^{-5}	0.40	
VPD x Relative humidity*	-9.146×10^{-5}	0.95	

Table 7. continued

Fertilized treatment, lower crown position

Parameter	Estimate	P-value	Model R^2
Growing season		n=230	
Intercept*	0.8106	0.0001	0.42
Log(VPD)*	2.606	0.0001	
VPD^2*	-0.01923	0.0008	
VPD x PAR*	-2.961×10^{-5}	0.53	
VPD x Relative humidity*	-0.009146	0.0001	
Non-growing season		n=168	
Intercept*	-0.1602	0.036	0.48
Log(VPD)*	-1.330	0.0001	
VPD^2*	0.2474	0.0001	
VPD x PAR*	3.438×10^{-4}	0.0001	
VPD x Relative humidity*	0.002071	0.15	

Fertilized treatment, upper crown position

Parameter	Estimate	P-value	Model R^2
Growing season		n=240	
Intercept*	0.5968	0.0001	0.42
Log(VPD)	0.06801	0.87	
VPD^2	-2.277×10^{-5}	0.998	
VPD x PAR	1.337×10^{-4}	0.0001	
VPD x Relative humidity*	-0.001174	0.47	
Non-growing season		n=172	
Intercept*	0.03249	0.77	0.34
Log(VPD)	-0.7031	0.0065	
VPD^2	0.1398	0.0029	
VPD x PAR	1.412×10^{-4}	0.0003	
VPD x Relative humidity*	0.002777	0.18	

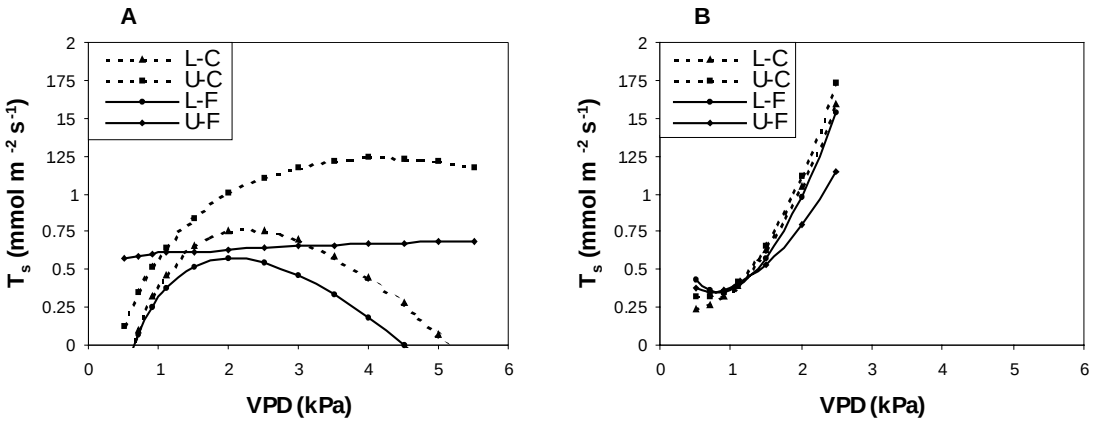

Figure 18: Predicted transpiration in relation to VPD for the growing season (A) and the non-growing season (B). L = lower third of crown, U = upper third of crown, C = control stands, F = fertilized stands.

transpiration during the growing season is highly variable depending on the fertilization treatment and crown position. Lower crown transpiration is greater in the control plots for a given VPD, reaching a predicted threshold in both fertilization treatments when VPD reaches about 2 kPa and transpiration achieves rates of about 0.75 and 0.5 mmol m^{-2} s^{-1} in control and fertilized plots, respectively. Predicted transpiration eventually ceases when VPD reaches 4.5 kPa in the fertilized stands and slightly over 5 kPa in the control stands. Predicted transpiration in the upper crown varies significantly depending on the fertilization treatment. Predicted transpiration in the upper crown of control stands reaches a maximum of about 1.25 mmol m^{-2} s^{-1} and slightly declines at higher VPD values. In fertilized stands, predicted transpiration is relatively unresponsive to increasing VPD, increasing by only about 0.11 mmol m^{-2} s^{-1} when VPD ranges from 0.5 kPa to 5.5 kPa.

Models for all fertilization treatment and crown position combinations within the non-growing season yield more similar predicted rates. This is evident when transpiration is plotted against VPD (Figure 18). Predicted transpiration rates for all fertilization treatment and crown position combinations increase with increasing VPD and do not meet a threshold within the range of VPD values recorded during non-growing season measurements. Both crown positions in the control stands appear to have slightly greater rates than those predicted in fertilized stands at VPD values greater than 1 kPa. Predicted maximum transpiration is achieved at the highest VPD value in the range (2.5 kPa), reaching over 1.5 mmol m^{-2} s^{-1} in the control stands and the lower crown of fertilized stands, and about 1.15 mmol m^{-2} s^{-1} in the upper crown of fertilized stands.

At least two (of five) parameter estimates statistically differ between seasons for every fertilization treatment and crown position combination, indicating that all models are different between the growing and non-growing seasons ($p<0.1$) (Table 7). This is illustrated in Figure 18 and more clearly in Figure 19. Predicted transpiration rates are greater for all fertilization treatment and crown position combinations during the non-growing season when VPD exceeds 1.5 to 2.0 kPa. Predicted transpiration is negatively affected by VPD at high values during the growing season (except in the upper crown of fertilized stands), but predicted rates during the non-growing season increase almost exponentially when VPD ranges from 1.5 to 2.5 kPa. Seasonal comparisons suggest that

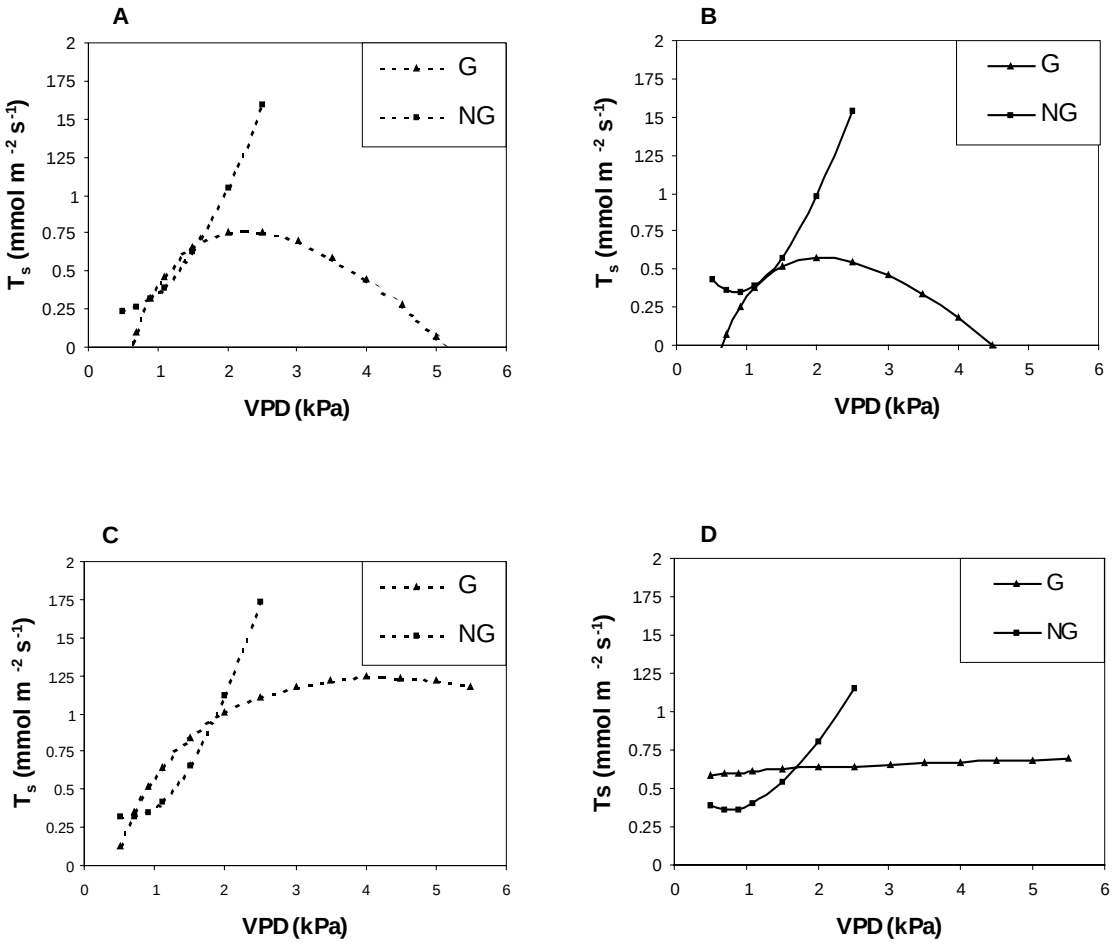

Figure 19: Predicted transpiration in response to VPD for the growing season and non-growing season for each fertilization treatment and crown position combination. Lower third of crown and control stands (A), lower third of crown and fertilized stands (B), upper third of crown and control stands (C), upper third of crown and fertilized stands (D). Growing = growing season (April – October), NG = non-growing season (November – March).

transpiration is less regulated during the non-growing season while water limitations during the growing season may encourage water conservation at higher VPD values.

January and July Intensive Measurement Periods

Transpiration prediction models for the January and July intensive measurement periods differ from prediction models for the non-growing and growing seasons, respectively (Table 8). At least one parameter estimate is significantly different between the January intensive and non-growing season models for all fertilization treatment and crown position combinations, except for upper crown in fertilized stands ($p<0.1$). Within the January intensive measurement period, models for fertilization and crown position combinations are statistically similar. Only the parameter estimate VPD x PAR is significantly lower in lower crown positions from both treatments ($p<0.1$). These differences are illustrated in Figure 20, which shows how predicted transpiration relates to VPD for both the January intensive measurement period and the entire non-growing season. Unlike the prediction models for the non-growing season, the model developed from January intensive measurements predicts little change in transpiration with increasing VPD. In fact, within the range of VPD values, the greatest predicted change in transpiration is about 0.16 mmol m^{-2} s^{-1}. The greatest predicted change in transpiration within the same VPD range for the entire non-growing season is greater than 1.25 mmol m^{-2} s^{-1}.

Models developed for the July intensive measurement period and the entire growing season differ as well. One or more parameter estimates for all fertilization treatment and crown positions significantly differ between the July model and the model for the entire growing season, indicating that predicted responsiveness to the environment is different. Upper crowns in fertilized stands respond entirely negatively to increasing VPD during July, but the model for the entire growing season predicts a slightly positive response to VPD (Figure 21). Predicted transpiration in the lower crown of fertilized stands reaches a maximum of 1.5 mmol m^{-2} s^{-1} in the July intensive model, but attains only a rate slightly greater than 0.5 mmol m^{-2} s^{-1} based on predictions for the entire growing season. The predicted transpiration rates in the lower crown of control stands

Table 8: Transpiration parameter estimates for common prediction models, corresponding p-values, and model R^2 values for the January and July intensive measurement periods for all fertilization treatment and crown position combinations. Stars next to parameters indicate estimates differ significantly between intensive measurement periods (P<0.1).

Control treatment, lower crown position

Parameter	Estimate	P-value	Model R^2
January		n=80	
Intercept	0.6031	0.041	0.67
Log(VPD)	1.315	0.058	
VPD	-0.07025	0.31	
VPD x PAR*	3.870×10^{-4}	0.0005	
VPD x Relative humidity	0.004581	0.30	
July		n=75	
Intercept	1.292	0.0025	0.58
Log(VPD)	2.214	0.066	
VPD^2	-0.03582	0.26	
VPD x PAR*	0.001005	0.013	
VPD x Relative humidity	-0.01449	0.015	

Control treatment, upper crown position

Parameter	Estimate	P-value	Model R^2
January		n=79	
Intercept*	0.5337	0.13	0.68
Log(VPD)*	0.8331	0.29	
VPD^2	0.06545	0.39	
VPD x PAR*	5.595×10^{-5}	0.10	
VPD x Relative humidity	-0.003745	0.50	
July		n=80	
Intercept*	2.1306	0.0001	0.69
Log(VPD)*	4.877	0.0027	
VPD^2	-0.06318	0.70	
VPD x PAR*	2.197×10^{-4}	0.0007	
VPD x Relative humidity	-0.02068	0.0041	

Table 8. continued

Fertilized treatment, lower crown position

Parameter	Estimate	P-value	Model R^2
January		n=79	
Intercept	0.1339	0.69	0.60
Log(VPD)	0.06417	0.94	
VPD^2	0.04062	0.59	
VPD x PAR*	3.001×10^{-4}	0.0001	
VPD x Relative humidity	0.001674	0.75	
July		n=71	
Intercept	0.7475	0.023	0.53
Log(VPD)	1.192	0.22	
VPD^2	-0.03151	0.19	
VPD x PAR*	8.5×10^{-4}	0.0057	
VPD x Relative humidity	-0.008293	0.072	

Fertilized treatment, upper crown position

Parameter	Estimate	P-value	Model R^2
January		n=80	
Intercept	0.01191	0.98	0.53
Log(VPD)	-0.2154	0.85	
VPD^2	0.06869	0.52	
VPD x PAR*	9.162×10^{-5}	0.058	
VPD x Relative humidity	0.005952	0.46	
July		n=80	
Intercept	1.253	0.014	0.47
Log(VPD)	-0.3273	0.83	
VPD^2	-0.06144	0.10	
VPD x PAR*	4.088×10^{-4}	0.0002	
VPD x Relative humidity	-0.008290	0.23	

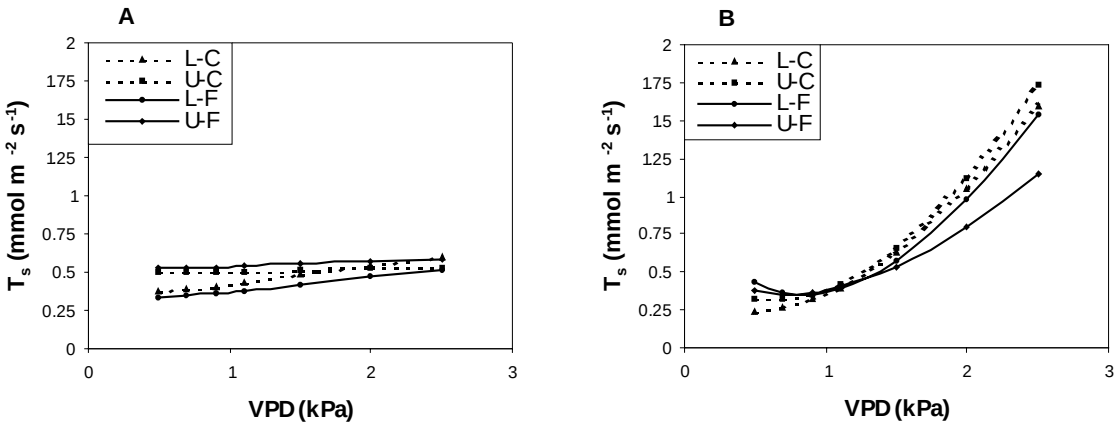

Figure 20: Predicted transpiration in relation to VPD for the January intensive measurement period (A) and the entire non-growing season (B). L = lower third of crown, U = upper third of crown, C = control stands, F = fertilized stands.

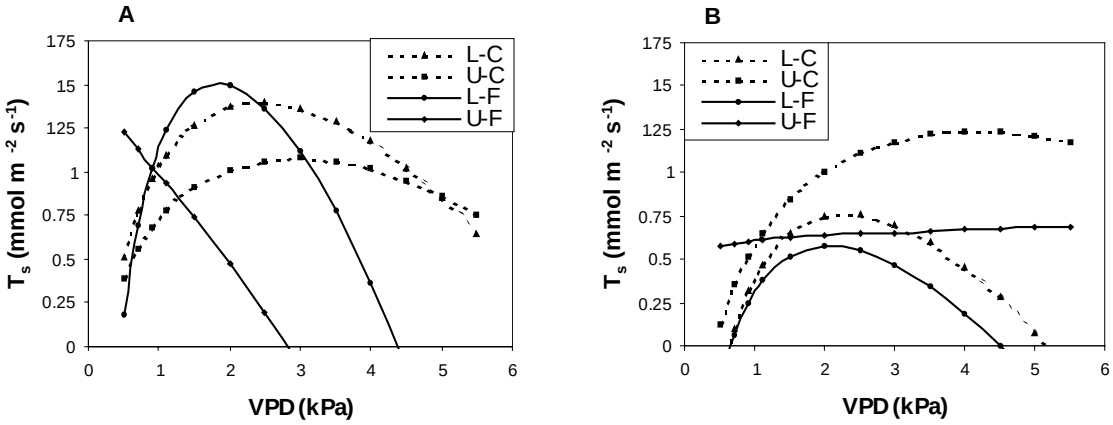

Figure 21: Predicted transpiration in relation to VPD for the July intensive measurement period (A) and the entire growing season (B). L = lower third of crown, U = upper third of crown, C = control stands, F = fertilized stands.

are generally higher than rates in the upper crown of control stands for the July intensive model. This trend is reversed in the prediction model for the entire growing season. Lower crown transpiration in control stands are predicted to decline at high VPD values for both the intensive and seasonal model; however, the model for the entire growing season predicts an eventual cessation in transpiration.

Within the July intensive period, only the prediction model for the lower crown of fertilized stands is significantly different from the other models. These differences are evident when predicted transpiration is plotted against VPD for both the July intensive measurement period and the entire growing season (Figure 21). Predicted transpiration in the lower crown of fertilized stands immediately drops with any increase in VPD. Other models indicate that transpiration increases with increasing VPD until a threshold is reached. In the upper crown of fertilized stands the threshold is reached around 2 kPa, followed by a steep decline and finally a complete halt in transpiration when VPD approaches 4.5 kPa. In the control stands, the threshold is reached at a VPD of approximately 2.5 kPa and 3 kPa in the lower and upper crowns, respectively. Predicted transpiration does not cease, however. In summary, intensive and seasonal transpiration prediction models highly differ, suggesting that the use of seasonal models to predict transpiration for a single day may be unreliable.

Statistical comparisons between July and January intensive model parameter estimates for each fertilization treatment and crown position combination show that at least one variable between models is significantly different ($p>0.1$) (Table 8). Predicted transpiration in January is much less responsive to VPD in comparison to July (Figure 22). As previously mentioned, predicted transpiration in July declines at high VPD values after reaching a threshold (except in the upper crown of fertilized stands which only declines with increasing VPD).

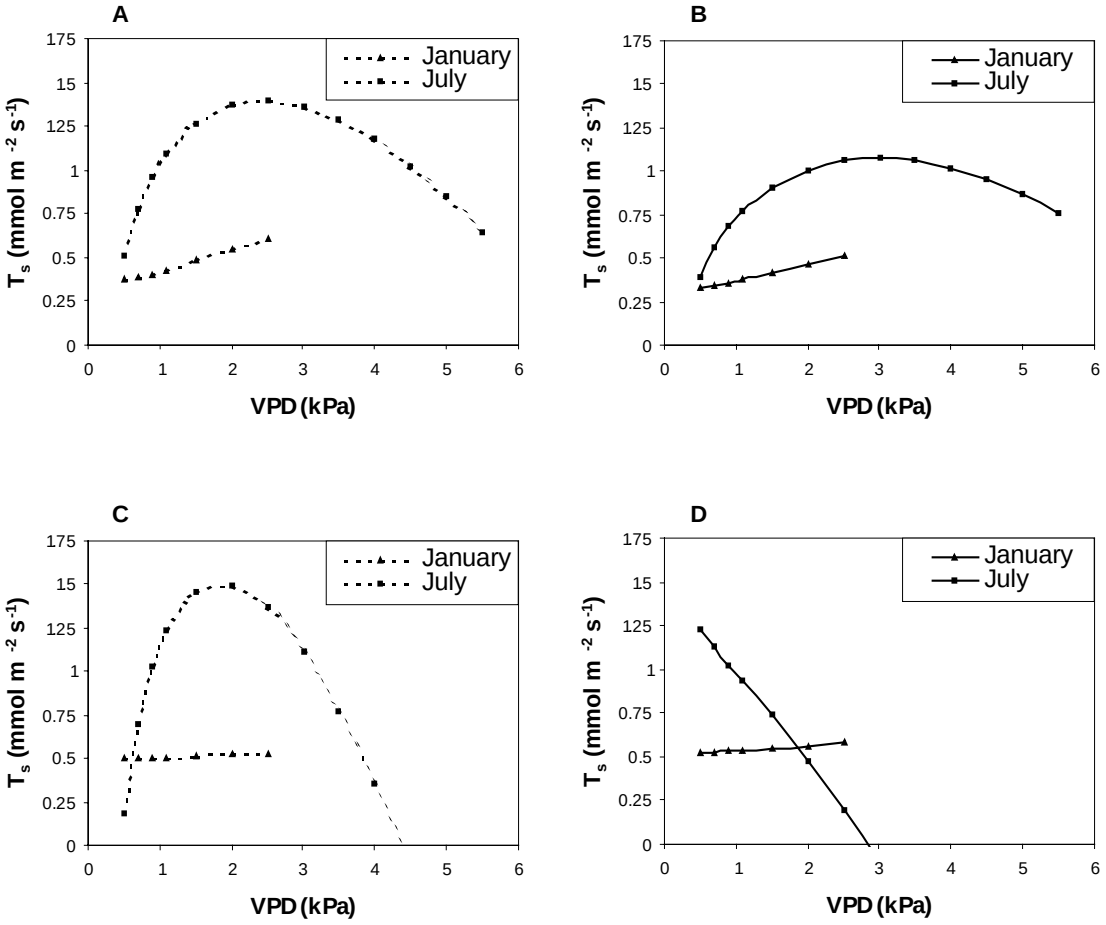

Figure 22: Predicted transpiration in response to VPD for the July and January intensives for each fertilization treatment and crown position combination. Lower third of crown and control stands (A), lower third of crown and fertilized stands (B), upper third of crown and control stands (C), upper third of crown and fertilized stands (D).

CHAPTER 5. DISCUSSION

Seasonal Trends in Gas Exchange

Seasonal gas exchange trends reveal that monthly differences in mean rates exist between both fertilized and control stands and lower third and upper third crown foliage. Data from this study show that mean photosynthesis was generally higher in the control stands during the growing season, although rates were only significantly greater than fertilized stands during April and May (Figure 1). Mean rates were greater in the fertilized plots during December, January, and February, but only significantly greater in February ($p<0.1$).

There are several possible explanations for seasonal differences in photosynthetic capacities between fertilized and control plots. One explanation involves the potential correlation between foliar nitrogen content and photosynthesis rates. Previous studies have conflictingly reported that both foliar nitrogen concentrations in conifers is highly correlated with photosynthesis while others found little or no correlation. Mitchell and Hinckley (1993) found that in Douglas fir photosynthetic rates and foliar nitrogen concentrations were positively correlated. In Scots pine seedlings, photosynthesis was strongly correlated with foliar nitrogen content only during the growing season (Vapaavuori 1995). Schoettle and Smith (1999) reported a weak relation between foliar nitrogen and photosynthesis in lodgepole pine, except in young leaves. However, no differences in photosynthesis were found in mature slash pine foliage that had been fertilized (Teskey *et al.* 1994). If a positive correlation between foliar nitrogen content and photosynthesis existed in loblolly pine during this study, one explanation for different mean photosynthesis rates involves the potential dilution of nitrogen that could occur in foliage from fertilized stands during the growing season, when young leaves are maturing. Data collected from the same stands in which this study was conducted show that leaf area indexes were approximately 50-100% greater in fertilized stands compared to control stands from 1992 through 1995 (Albaugh *et al.* 1998). Since more nutrients are required to support greater amounts of foliage, fertilized stands may actually have lower leaf nitrogen percentages during the growing season when these nutrients are being assimilated into compounds required by the rapidly growing foliage. However, during

the non-growing season when nitrogen is no longer required for growth, photosynthesis rates would be higher in fertilized stands as exemplified in this study.

Previous reports from loblolly pine studies suggest that there is no correlation between photosynthesis rates and foliar nitrogen concentrations. Zhang *et al.* (1997) reported that although nitrogen fertilization increased leaf nitrogen in loblolly pine, there was no increase in photosynthesis rates or quantum yield. They concluded that unless leaf chlorophyll is limiting, nitrogen fertilization would not offset the effects of low-light acclimation on photosynthesis. Results from this study support the notion that there is little or no correlation between foliar nitrogen content and photosynthesis rates in loblolly pine. Actual foliar nitrogen content (in percent dry weight) was determined for both control and fertilized stands (Figure 2A). Results indicate that foliar nitrogen content was statistically greater in fertilized stand for all months examined ($p<0.1$). If nitrogen content is related to differences in photosynthesis rates between control and fertilized stands, rates should be higher in fertilized stands for all months in which foliar nitrogen content was determined. However, this is not the case. Also, statistical analysis revealed that nitrogen was not a significant component of photosynthesis prediction models. When photosynthesis was correlated with only nitrogen on a monthly basis (data not shown), the relationship was only significant during October, when foliar nitrogen content was positively correlated with photosynthesis ($p=0.08$). Similar results were reported in another loblolly pine study conducted in Louisiana. Tang *et al.* (1999) found that fertilized trees had lower mean photosynthesis, transpiration, and conductance than unfertilized trees from June through November. This is consistent with data presented in this study (with the exception of November). Interestingly, a study conducted in the same stand as the one in which this study was performed found that at nine years old (five years younger), mean monthly photosynthesis at saturating light levels (PAR>1600 μmol m^{-2} s^{-1}) was greater in fertilized stands compared to control stands from January through September (Murthy *et al.* 1997). This would be expected during the non-growing season based on both mean monthly rates and predicted rates (Figure 13B), but this would not be expected during the growing season according to monthly rates or predicted rates (Figure 13A). Potential reasons for differences in photosynthetic

capacities found in the study performed by Murthy *et al.* and in the current study are addressed later.

Other potential explanations regarding the difference in photosynthesis rates between fertilized and control stands are less conclusive. Tang *et al.* (1999), who measured photosynthesis at ambient light in the crown, concluded that foliage in control stands had higher average rates because fertilized stands had more foliage, which resulted in leaf shading. However, this conclusion is not consistent with results presented in this study since photosynthesis was measured at the same light intensities in both fertilized and control plots. Furthermore, models developed in this study predict that fertilized and control foliage respond differently to PAR, suggesting that physiological differences rather than environmental variation is responsible for different rates.

Zhang *et. al* (1997) proposed another explanation for why a relationship between photosynthesis and foliar nitrogen concentration was not found in their study, pointing out that fertilized trees may not be nitrogen limited. Additional foliar nitrogen probably contributes to increased levels of carbon fixation enzymes such as ribulose 1,5-bisphosphate carboxylase oxygenase (RuBISCO) (Field and Mooney 1986). However, if light absorption compounds (light harvesting complexes) or other necessary photosynthetic structures are limiting then additional nitrogen may not improve photosynthesis status. In this study, fertilized trees still may be diluting their resources during the growing season by investing in larger amounts of foliage production, which would explain lower photosynthesis rates during the growing season. However, nitrogen limitations do not explain differences in photosynthesis between control and fertilized stands.

Previously published reports, which examined the same stand as this study, indicate that the seasonal differences between control and fertilized stands found in this study were not present after the stand was initially fertilized. Data from a study by Murthy *et al.* (1997), which was collected two years after fertilization treatments began (9 years old), suggest that foliage from fertilized stands had higher photosynthetic capacities than control foliage during the growing season. The percent difference in leaf area indexes (LAI) increased from 1992 to 1996 in the stand from the current study (Albaugh *et al.* 1998). Nine months after nutrient additions, a more rapid increase in LAI

was apparent in fertilized stands, but the percent difference between control and fertilized stands during 1995 and 1996 did not increase as much relative to the first two years after fertilization. Initially fine root biomass was greater in fertilized stands, but was greater in control stands when sampled from 1993 to 1996. Coarse root biomass consistently increased in fertilized stands for all years sampled after fertilization. These results combined strongly indicate that nutrient additions may have increased photosynthesis in fertilized stands on a per leaf area basis at one point in order to provide the photoassimilates necessary for the additional leaf area in fertilized stands. This potentially explains why Murthy *et al.* (1997) found that fertilized trees had higher photosynthesis rates in 1994. An initial rapid increase in fine root biomass in fertilized stands allowed for the maximum uptake of nutrients to support higher photosynthesis rates. Since the percent differences in LAI became relatively stable in 1995 and 1996, photosynthesis rates per leaf area may have leveled or decreased since supplementary carbohydrate pools were no longer required to support rapid leaf growth. Thus, after maximum increases in leaf areas were achieved in fertilized stands, photosynthesis may have been downregulated, which is consistent with data presented from this study. Photosynthetic capacities have been shown to adjust to changing demands in carbohydrate sinks such as roots and fruits (Kramer and Kozlowski 1979), and also to changes in foliage growth (Boltz *et al.* 1986). Once greater leaf areas were achieved in the current stand, downregulation of photosynthesis on a per leaf area basis may have allowed fertilized foliage to actually improve energy efficiency since enhanced rates were no longer useful for foliage production. However, overall crown photosynthesis of fertilized trees remains greater than control trees since more photosynthetic machinery is in place. Foliage from control stands on the other hand may be required to maximize photosynthetic efficiency by optimizing photosynthesis on a per leaf area basis since the opportunity to enhance overall crown photosynthesis via increased leaf area is clearly nutrient limited. This trend of initially upregulating photosynthesis after nutrient additions in order to maximize leaf area has been demonstrated in other species. In a study conducted on tall fescue (*Festuca arundinacea*), the authors concluded that nitrogen fertilization affected shoot growth by changing carbon partitioning which resulted in both faster leaf area development and greater light interception (Belanger *et*

al. 1994). Teskey *et al.* (1994) found that in mature slash pine, LAI generally increased in fertilized stands, but photosynthesis on a per leaf area basis was not substantially affected; again, suggesting that rates may have initially been higher in fertilized stands in order to produce the photosynthate necessary for higher leaf areas.

In summary, this study suggests that nutrient additions in loblolly pine did not increase the efficiency of the photosynthetic machinery during the course of this study; fertilized trees simply were able to produce and maintain more foliage than unfertilized trees. Results imply that mean net productivity was more related to leaf biomass rather than photosynthetic efficiency during the course of this study. Linear relationships between leaf biomass and mean net productivity in loblolly pine have been previously presented (Teskey *et al.* 1987). However, net photosynthesis on a per leaf area basis must have been greater at some point after fertilization in order to provide the photoassimilate necessary for improved leaf area.

Mean monthly conductance (Figure 3A) and transpiration (Figure 4A) rates basically mirrored trends in photosynthesis between fertilized and control stands. This is probably due mostly to the fact the conductance largely dictates the carbon available for photosynthesis. Since transpiration is defined as conductance x a driving force (VPD), transpiration will closely resemble conductance when VPD is fairly constant.

Variable light intensities explain a majority of the differences in photosynthesis rates between the lower and upper thirds of crowns. Light is well documented as the major environmental driver of photosynthesis, however physiological differences between light and shade leaves also affect photosynthetic capacity. Table 2 shows the mean monthly environmental conditions, including PAR for the lower and upper third of crowns. Clearly, photosynthesis rates are closely associated with differences in mean light levels between the upper and the lower crowns and this is reflected in mean photosynthesis rates. Also, prediction models for photosynthesis show that physiological differences exist between foliage from the lower and upper crowns. All prediction models indicate that lower crown foliage is less responsive to light than upper crown foliage at the same PAR, except at lower light levels during the growing season (Figure 13A). During the growing season, lower crown foliage has a greater predicted initial quantum yield than the upper crown foliage.

Mean foliar nitrogen content was generally greater in the upper third of the crown compared to the lower third, although nitrogen content was only significantly greater in the upper third of the crown in July and October ($p<0.1$) (Figure 2B). This is consistent with general characteristics of sun leaves compared to shade leaves.

Conclusions concerning variation in crown gas exchange reported by Tang *et al.* (1999) are consistent with the results of this study. Not surprisingly, monthly mean photosynthesis, conductance, and transpiration from June to November was significantly greater in the upper crown compared to the lower crown ($p<0.1$). Differences in gas exchange were attributed mainly to variation in light intensity and temperature within the crown. A study by Gravatt *et al.* (1997) which examined temporal and spatial patterns of net photosynthesis in thinned 12-year-old loblolly pine stands similarly concluded that upper crown levels within the fertilized and unfertilized plots had significantly higher light levels and photosynthesis rates than lower crown foliage.

Water use efficiency was also calculated and statistically compared between control and fertilized stands and lower and upper thirds of crowns (Figure 5). Results indicate that water use efficiency generally did not statistically differ between fertilization treatments, but does differ between crown positions for seven months ($p<0.1$). Mean monthly water use efficiency was generally greater in fertilized stands, but was significantly greater in fertilized stands only during March and July. Although photosynthesis is higher during these months in the control stands, transpiration is disproportionally higher which explains lower water use efficiencies in the control stands. One explanation for this difference is that fertilized trees more tightly regulate water spending since they have greater amounts of foliage and generally the same soil water status as trees in control stands. This concept is discussed further in "Predicted Gas Exchange Trends". These results are consistent with those reported in a Douglas-fir study which found that water use efficiency did not significantly differ between shoots containing high levels of nitrogen and low levels of nitrogen (Mitchell and Hinckley 1993).

Overall, mean monthly gas exchange rates appear to be influenced mostly by the immediate environment, although acclimation does occur and optimum environmental conditions are known to shift throughout the year in loblolly pine (Boltz *et al.* 1986,

Bongarton and Teskey 1986, Drew and Ledig 1981). Although prediction models developed from this study show that foliage during the growing season is capable of reaching higher photosynthesis rates, mean monthly photosynthesis rates mostly reflect the average PAR levels during measurements. For example, mean PAR levels during measurements in June for both crown positions were only higher than those recorded in December (Table 2). The low light levels are reflected in the comparatively low rates in June, reaching slightly less than 2.5 μmol m^{-2} s^{-1} in the upper third of crowns. The highest mean monthly PAR levels were recorded in September, when mean photosynthesis in the upper crown attained an average of slightly greater than 4.25 μmol m^{-2} s^{-1}.

Diurnal Trends in Gas Exchange

Diurnal trends indicate that there are few significant differences in gas exchange between fertilization treatments during both January (Figure 6) and July (Figure 7). However, consistent with seasonal trends, gas exchange was generally significantly greater in the upper third of the crown compared to the lower third of the crown for January (Figure 8) and July (Figure 9). Lowest photosynthesis rates were recorded at dawn and dusk for both months, which supports the notion that light most significantly influences photosynthesis rates.

Gas exchange rates fluctuated throughout the sequential measurement days during both January and July due to the rapid changes in environmental conditions that occurred within a single day. Also, physiological differences between foliage from the four treatment combinations resulted in the variable responses to the environment displayed in the prediction model response curves. Maier and Teskey (1992) similarly reported that both internal and external factors influenced seasonal and diurnal trends in white pine foliar gas exchange. Although light appears to be the most influential driver of photosynthesis, other environmental conditions clearly affected photosynthesis throughout the measurement days during both January and July. Conductance and transpiration also affected photosynthesis throughout the measurement days. Conductance fluctuated less during January than during July. In January, transpiration closely followed conductance on the first day and photosynthesis on the second

measurement day, suggesting that little stomatal control was exerted on the second day (Figures 6, 8). This shows that transpiration was partially driven by VPD on the second day of measurements in January. Mean VPD values on the second measurement day in January also support this idea (Figure 11).

July photosynthesis rates appear to be influenced in part by water status. In July, photosynthesis peaked around 9 a.m. on both days, probably due to increasing water limitations later in the day. Photosynthesis dropped by as much as 40%, relative to the peak rate for the day, during the afternoon (Figure 9B). This is especially surprising given that PAR levels were actually greatest in the afternoon on the second day of measurements, after the drop in photosynthesis rates occurred (Figure 11). When photosynthesis peaked around 9 a.m., PAR levels were 1200 μmol m^{-2} s^{-1}. However, during the next two measurement periods a significant drop in photosynthesis occurred when PAR levels were 1800 μmol m^{-2} s^{-1} and 1700 μmol m^{-2} s^{-1}, respectively. These limitations are manifested in declining conductance rates in the afternoon on both July measurement days, which resulted in decreased C_i. Conductance closely tracked photosynthesis on both days. On the first measurement day in July, a brief period of heavy rain around 2 p.m. appears to have allowed both conductance and photosynthesis to rebound slightly. Corresponding PAR values support this idea since mean photosynthesis was slightly greater after the rain even though PAR was 1200 μmol m^{-2} s^{-1} in the upper crown prior to the rain and only 700 μmol m^{-2} s^{-1} after the rain (Figure 9A). This is probably partially due to an increase in water potential after the rain event from –19 bars to –15 bars and a decline in VPD. Leaf water potential throughout the day varied during both January and July, but was clearly more negative in July (Figure 10, 11). Mean water potential was –15 bars or less during half of the measurement periods in July while water potential never exceeded –14.5 bars during January measurements. Although water potential is not a variable in the common models for January and July intensive photosynthesis, water potential x PAR interaction was a significant variable ($p<0.1$) in July models for fertilized stands only. Using the parameter estimate for water potential x PAR from a preliminary stepwise model for the upper crown of fertilized stands and actual environmental data from the second day of measurements in July, the change in photosynthesis with changing water potential and

PAR was calculated. According to the model, a significant drop in photosynthesis is predicted as water potential becomes more negative throughout the day. In the morning, when PAR was only 50 μmol m^{-2} s^{-1} and xylem water potential was only slightly less than -3 bars, virtually no drop in photosynthesis is predicted. However, in the afternoon when PAR was 1800 μmol m^{-2} s^{-1} and water potential was less than -17 bars, a decrease in photosynthesis of 1.2 μmol m^{-2} s^{-1} is predicted. Therefore, water potential alone may have limited photosynthesis during July intensive measurements, at least in fertilized plots.

Transpiration varied little on the first day of measurements in July, but increased considerably throughout the second day as conductance simultaneously decreased. Consistent with models developed from this study, this pattern indicates that while stomates were closing VPD must have increased sufficiently throughout the day in order to overcome increasing stomatal resistance. Mean VPD values throughout both days in July support this reasoning. VPD values ranged from 0.8 kPa during the 6:30 a.m. measurement period to a mean value of 4.6 kPa for the 2:45 p.m. measurement period. (Figure 11). Increasing VPD coupled to increasingly negative xylem water potential may partially explain the drop in photosynthesis on the second day in July. Water potential alone is not responsible for this decrease since water potential values were actually most negative on the first day of measurements, prior to the rain event. Mean VPD values in January exhibited a more limited range throughout the day compared to July. As with the July intensive measurement period, the slight increase in VPD on the second day of measurements in January may partially explain the increase in transpiration throughout the day.

The diurnal trends in loblolly pine gas exchange discussed above are generally supported in the literature. Loblolly pine photosynthesis reportedly has a relatively small response to VPD, except at the highest VPD values (Teskey *et al.* 1987). However, in two separate experiments, a decline in loblolly pine photosynthesis during the growing season corresponded to significant increases in VPD and a decline in leaf water potential (Teskey *et al.* 1987, Fites and Teskey 1988). Both studies concluded that greater stomatal limitations at high VPD values caused lower C_i values, resulting in decreased carbon fixation. As in this study, both studies consistently show that net photosynthesis

peaked in the morning during the growing season. Several studies have shown that extremely negative water potentials alone may significantly limit photosynthesis. Dang *et al.* (1997) found that photosynthesis in black spruce decreased linearly with decreasing water potential while jack pine reached a threshold when xylem water potential reached –10 bars. Maier and Teskey (1992) reported that light and water status were the most limiting environmental variables to photosynthesis in white pine. Teskey *et al.* (1986) found that loblolly pine seedlings under laboratory conditions reached a threshold when water potential is reduced below –10 bars. Light saturated photosynthesis was 6 μmol m^{-2} s^{-1} at –10 bars, but decreased to 2 μmol m^{-2} s^{-1} when xylem pressure potentials were reduced to –15 bars. However, data from this study show that loblolly pine in the field is capable of maintaining photosynthesis rates of at least 3.5 to 4 μmol m^{-2} s^{-1} when leaf xylem water potential is –15 bars or less.

Mean maximum photosynthesis rates differed between January and July by about 1.5 μmol m^{-2} s^{-1}. However, PAR levels in the upper crown were only 1200 μmol m^{-2} s^{-1} in July when photosynthesis reached a maximum of about 6 μmol m^{-2} s^{-1} (Figure 9B) while PAR levels were 1800 μmol m^{-2} s^{-1} when rates peaked at 4.5 μmol m^{-2} s^{-1} on the second day in January (Figure 8B). Photosynthesis during the intensive measurement periods in January closely tracked changing PAR. During July this is not the case; exceptions are probably the result of water limitations as discussed above. Differences in maximum net photosynthesis rates between January and July are also attributable to seasonal acclimation displayed by loblolly pine. Boltz *et al.* (1986) found higher rates during the growing season compared to the non-growing season. Significant differences among photosynthesis prediction models presented in the study also suggest that seasonal acclimation occurs. A maximum photosynthesis rate of 6 μmol m^{-2} s^{-1} in July is consistent with rates reported by Teskey *et al.* (1986) in loblolly pine seedling studies conducted in a control setting. When irradiance was 1500 μmol m^{-2} s^{-1}, VPD was low, temperature was 25°C, and plants were well-watered, net photosynthesis ranged from approximately 5 to 6 μmol m^{-2} s^{-1}.

Mean ambient temperatures were lower in January than July, ranging from about 10-20°C during January measurements and from 25-40°C in July (Figures 10, 11). These ranges exceed the optimum seasonal temperatures reported for loblolly pine seedlings

from the coastal plain of North Carolina. Drew and Ledig (1981) concluded that optimum temperatures for net photosynthesis shifted with seasonal changes in temperature, reaching 25°C in midsummer and decreasing to 10°C in midwinter. Although temperatures were above optimum for photosynthesis during both January and July measurement periods, the stepwise procedure used to formulate common prediction models shows that temperature is not significant in models or contributes little to the total model R^2, suggesting that temperature did little to affect photosynthesis rates. However, temperatures below the optimum could potentially reduce photosynthesis rates, especially when temperatures are below freezing, by lowering conductance rates (Teskey *et al.*, 1987). Since temperatures during measurements did not achieve freezing, temperature may not have significantly affected gas exchange.

Predicted Gas Exchange Trends

Photosynthesis

Statistical comparisons of both photosynthesis and transpiration model parameter estimates show that all fertilization treatment and crown position combinations statistically differ enough to justify separate models. Likewise, models for the same fertilization treatment and crown position combination are consistently different between both the growing and non-growing seasons and the January and July intensive measurement periods. Therefore, separate models for all fertilization treatments and crown position combinations for both the growing and non-growing seasons are required to more accurately predict gas exchange. These results confirm that the environment does not solely dictate changes in gas exchange, suggesting that physiological differences exist between foliage from the lower and upper thirds of crowns and foliage from fertilized and control stands. Also, seasonal acclimation, which is discussed later, must be partially responsible for differences between growing and non-growing season models.

Predicted light-response curves illustrate differences in photosynthesis between fertilization treatments, crown positions, and seasons (Figure 12A, 13, 14, 15, 16, 17). When predicted net photosynthesis is plotted against PAR, predicted responses by lower and upper crown foliage resemble typical light-response curves corresponding to shade

and sun foliage, respectively. These differences are most apparent among prediction models for the lower and upper thirds of crowns during the growing season. Consistently, upper crown foliage in both fertilized and control stands has higher predicted maximum photosynthesis rates, higher predicted respiration, higher predicted light compensation point, and lower predicted initial quantum yield. Statistical comparisons of parameter estimates for the Ln(PAR) and of the y-intercept confirm that significant differences in light responsiveness and predicted respiration exist between the lower and upper crown foliage.

Predicted photosynthesis rates are within the range of reasonable values according to previously published data. Light-response curves performed on seedlings in a controlled laboratory environment exhibited similar responses to irradiance as those predicted in this study (Teskey *et al.* 1986). Photosynthesis prediction models based on data from the entire year predict that rates in the upper crown foliage will reach 1 μmol m^{-2} s^{-1}, 2 μmol m^{-2} s^{-1}, 3 μmol m^{-2} s^{-1}, and 4 μmol m^{-2} s^{-1} when PAR is equal to approximately 150 μmol m^{-2} s^{-1}, 300 μmol m^{-2} s^{-1}, 550 μmol m^{-2} s^{-1}, and 1400 μmol m^{-2} s^{-1}, respectively. Actual light response curves in the seedlings produced similar results at the first three light levels, achieving rates of 1 μmol m^{-2} s^{-1}, 2 μmol m^{-2} s^{-1}, 3 μmol m^{-2} s^{-1}, and 4 μmol m^{-2} s^{-1} when PAR is equal to approximately 200 μmol m^{-2} s^{-1}, 300 μmol m^{-2} s^{-1}, 500 μmol m^{-2} s^{-1}, and 800 μmol m^{-2} s^{-1}, respectively.

Photosynthesis prediction models based on data from the entire year show that fertilized foliage is more negatively affected by increasing VPD than control foliage. The most reasonable explanation for this behavior is that larger leaf areas in fertilized trees result in the need to conserve more water on a per leaf area basis since greater leaf areas provide a larger transpiration surface and therefore potential for greater water loss. Assuming the soil in both the control and fertilized stands has approximately equal moisture content, trees in fertilized stands must either increase the water absorption surface area (i.e. roots) or decrease the amount of water lost through transpiration. Trends from actual data taken during the July intensive measurement periods also suggest that photosynthesis in fertilized stands may be more negatively affected by increasing VPD. On the first day of measurements VPD was relatively stable, fluctuating by only about 1 kPa throughout the day. However, as previously discussed, VPD more than

tripled throughout the second day of measurements in July which could be characterized as a water limiting day with xylem water potentials less than –15 bars in the afternoon (Figure 11). Xylem water potentials were only slightly more negative in the afternoon on the first day of measurements, indicating that differences in gas exchange could at least partially be attributed to extreme differences in VPD between the two days. On the second day of July measurements, photosynthesis was inversely related to VPD in both the control and fertilized stands, but mean photosynthesis in fertilized stands was consistently lower (although not significant) throughout the day (Figures 7, 11). Conductance steadily decreased throughout the day in the fertilized stands and was significantly lower during the last two measurement periods. This decrease in conductance could partially be a response to VPD, in an effort to decrease water loss. Evidence for this is found in corresponding mean transpiration rates in fertilized stands, which leveled during the last two measurement periods, but continued to rise in the control stands. Since fertilized trees have a greater need to conserve water on a leaf area basis when water is limiting, mechanisms may exist via stomatal control to regulate and stabilize water loss. Although no distinction has been made concerning fertilized and unfertilized loblolly pine responses to water deficit in the literature, stomatal regulation in pines as a method to avoid water deficiencies is well documented in several species (Aphalo and Jarvis 1991, Bongarten and Teskey 1986, Dang et al 1997, Grantz 1990). However, Teskey *et al.* (1994) found no differences in gas exchange between fertilized and unfertilized mature slash pine when xylem water potential was low (less than -15 bars) and VPD was relatively high (up to 3.5 kPa), indicating that fertilization does not necessarily enhance stomatal control when water status is low. Attempts to model conductance in an effort to verify stomatal responsiveness to VPD and water potential were not successful due to significant unexplained variation among rates. Therefore, any correlation between conductance and the environment was not detected.

Predicted net photosynthesis in the upper and lower crowns of control stands is generally greater than in the same crown position in fertilized stands. Predicted net photosynthesis rates in the control stands are always higher during the growing season compared to the non-growing season (Figure 13). During the non-growing season, predicted rates are greater in control stands until PAR is greater than 1500 μmol m^{-2} s^{-1}.

These predicted trends are consistent with actual seasonal data discussed previously, suggesting that fertilized trees may dilute resources during the growing season since more foliage is present per tree in fertilized stands. During the non-growing season, predicted photosynthesis values in fertilized stands suggest that fertilized foliage is not light saturated when PAR is 2000 μmol m^{-2} s^{-1}. This also supports the notion that resources are less limiting in fertilized stands during the non-growing season, when foliar growth does not occur. Predicted trends suggest that fertilized stands during the non-growing season are not limited by carbon fixation rates, but rather by light. Because fertilized trees should have more resources available for maintenance processes rather than growth processes during the non-growing season, proteins involved in carbon fixation such as RuBISCO may be more abundant in fertilized trees during the non-growing season. However, the initially lower quantum yield in the upper crown of fertilized stands suggests that lower levels of light harvesting complexes (i.e. photosystem II) may be present in fertilized foliage during the non-growing season. RuBISCO concentrations have been linked to differences in photosynthesis rates between fertilized and non-fertilized plants (Fredeen *et al.* 1998, El Kohen and Mousseau 1994). Loustau *et al.* (1999) determined that in *Pinus pinaster*, the maximal velocity of carboxylation by RuBISCO was greater in trees that were not phosphorus limited. If control trees are phosphorus limited, this may explain differences in predicted saturated photosynthesis between fertilized and control trees during the non-growing season.

Other differences exist in predicted gas exchange trends between the growing and non-growing seasons (Figure 14). Predicted photosynthesis in the lower crown for both treatments is greater during the non-growing season. One possible explanation for this predicted trend is that higher total foliage during the growing season may have caused acclimation to occur in the lower crown, resulting in leaves that behaved more like shade leaves. If this is true, lower crown foliage should have similar predicted rates to the upper crown foliage during the non-growing season. In contrast, lower and upper leaves should perform more typically like shade and sun leaves during the growing season. As discussed earlier, this appears to be the case as evidenced by predicted rates in Figure 13. Zhang *et al.* (1997) studied acclimation to light intensities in loblolly pine, reporting that physiological changes occurred in foliage that was subjected to shading treatments.

Teskey *et al.* (1986) similarly concluded that shading caused lower rates late in the growing season. Other differences between upper and lower crown foliage are also apparent. In the upper crown, predicted quantum yield is initially higher and the light compensation point is lower during the non-growing season (Figure 13). Maximum photosynthesis and predicted respiration in both fertilization treatments is generally greater during the growing season. However, predicted photosynthesis rates converge when PAR is 2000 μmol m^{-2} s^{-1} in the upper crown of fertilized stands. Seasonal changes in maximum photosynthesis rates in loblolly pine are well documented (Boltz *et al.* 1986, Drew and Ledig 1981, Muthey *et al.* 1997, Teskey *et al.* 1986). Differences in quantum yield suggest that light harvesting complexes are more abundant in foliage during the non-growing season, while RuBISCO may be more limiting at higher light levels during the non-growing season compared to the growing season. Enzyme (i.e. RuBISCO) efficiency limitations due to lower temperatures during the non-growing season may be partially responsible for lower maximum rates compared to the warmer growing season (Nilsen and Orcutt 1996). Temperatures were lower during the non-growing season compared to the growing season in this study, supporting this idea (Table 2).

Conductance

Preliminary conductance prediction models were generally poor, having low R^2 values even when several variables were included in the model (Table 1). Common models for all fertilization and crown position combinations were difficult to develop since the primary variables were not "common" among all models. However, the variables selected through the stepwise procedure in SAS indicate that VPD, air temperature, and relative humidity drive conductance (although only weakly), which is consistent with numerous studies. The results are consistent with several other studies that found loblolly pine leaf conductance is relatively unresponsive to changes in the environment (Bongarten and Teskey 1986, Teskey *et al.* 1986, Teskey *et al.* 1987). Since the environment was not a good predictor of conductance, prediction models were not developed for conductance.

Transpiration

As with photosynthesis, common prediction models for transpiration differed statistically in virtually every comparison, indicating that separate models are required for all treatment and crown position combinations for both the growing and non-growing seasons. Differences among models are clearly illustrated when transpiration is plotted against VPD, the primary driver in all common models (Figure 12B, 18, 19, 20, 21, 22). Predicted transpiration rates differ highly among all models, suggesting that transpiration response surfaces are relatively variable. However, some predicted trends do exist among most models.

The prediction models for all fertilization treatment and crown position combinations based on data from the entire year exhibit predicted trends consistent with the idea that fertilized stands transpire less on a per leaf area basis (Figure 12). These trends support those ideas discussed in the "Photosynthesis" section concerning the need for fertilized trees to regulate water use on a per unit leaf area in order to maintain a healthy whole-tree water status.

Higher transpiration rates are predicted to occur in the control stands compared to the fertilized stands for each crown position for both the growing and non-growing seasons as well, although predicted transpiration is highly variable between the two seasons (Figure 18). During the growing season, rates are predicted to reach a threshold in the lower crown positions, eventually falling to zero at high VPD values. This is possibly in an effort to reduce water loss by allowing only the more photosynthetically efficient surfaces consisting of upper crown foliage to actively transpire and photosynthesize. In the upper third of the crown during the growing season a threshold is not clearly present. As previously discussed, a stomatal mechanisms may exist in order to limit water loss. During the non-growing season, transpiration is predicted to increase practically exponentially with VPD and predicted values are similar among all fertilization treatments and crown position combinations. During the non-growing season water was not as limiting as evidenced by water potential values (Table 2). Therefore, either the mechanism to control water loss was not triggered because the trees were generally not water stressed during the non-growing season or stomatal regulation is

lacking. Some evidence for temperature dependent changes in stomatal sensitivity have been noted in loblolly pine (Teskey *et al.* 1997).

Few transpiration prediction models exist in the literature based on field data for an entire year. However, actual data from loblolly pine studies present a similar range of rates as those predicted by models from this study. Teskey *et al.* (1986) found that under controlled conditions, transpiration ranged from about zero to almost 1.5 mmol m^{-2} s^{-1}, which is similar to the predicted range of zero to 1.75 mmol m^{-2} s^{-1} found in this study. Tang *et al.* (1997) reported mean rates higher than 2 mmol m^{-2} s^{-1} in the upper crown of loblolly pine. Actual data in laboratory settings shows that transpiration always increased with VPD (Bongarten and Teskey 1986, Teskey *et al.* 1986). However, data from this study suggest that this is not always the case during the growing season. Again, water limitations combined with rising VPD values in the field probably account for the predicted threshold and eventual cessation of predicted transpiration. Water potential is not in the common prediction models for photosynthesis probably only because rising VPD frequently corresponded to decreasing water potential values. This pattern is evident throughout both days during the July diurnal (Figure 11).

Seasonal and Diurnal Variation as Indicators of Acclimation

As demonstrated up to this point, both seasonal and diurnal variation exists in gas exchange partly due to the environment. In addition, unexplained seasonal variation in gas exchange may be due in part to acclimation events that occur over a longer period of time as the result of prolonged environmental stimuli (i.e. changes in photoperiod), and physiological processes governed by genetics (i.e. leaf growth). Although environmental variation is accounted for in the prediction models, acclimation reduces the explained variation since these processes are not accounted for in the models. Furthermore, acclimation affects how the environment influences gas exchange by causing shifts in optimal conditions (Drew and Ludig 1981). The extent of seasonal variation can be assessed by comparing the diurnal variation from representative days within a given season with data from the entire corresponding season, assuming that most acclimation events require several days or weeks to fully occur. A comparison of R^2 values from common models developed both from data taken during intensive measurements and

seasonal data indicates that seasonal acclimation did occur throughout the year of measurements. On average, model R^2 values are generally greater for both photosynthesis and transpiration models based on only diurnal data compared to models developed from the entire season (Tables 5, 6, 7, 8). For photosynthesis, average R^2 values of common models for the growing and non-growing seasons are 0.60 and 0.63, respectively; R^2 values of common models for July and January average 0.68 and 0.60, respectively. The lower average R^2 value for the January intensive diurnal are due to the low R^2 values of models for the lower crown. Model R^2 values for individual fertilization treatment and crown position combinations are as high as 0.64 for the seasonal models, but reach 0.79 in the intensive models. Transpiration model R^2 values reveal similar differences among seasonal and diurnal models. Average R^2 values of the growing and non-growing season models are 0.46 and 0.52, respectively, but are 0.62 and 0.56 for the July and January intensive models, respectively. Seasonal models for individual treatments include only one R^2 value greater than 0.6, but R^2 values are greater than 0.6 for three diurnal models. Other evidence for acclimation lies in the fact that model R^2 values are generally higher when models for fertilization treatment and crown position combinations are broken down into growing and non-growing season models rather than developed from data for the entire year even though the sample size is larger for models based on data from the entire year. Acclimation is also evident upon comparison of seasonal and diurnal predicted values for photosynthesis and transpiration (Figures 15, 16, 20, 21), since seasonal and diurnal predicted rates differ for the same environmental parameters. Thus, comparisons confirm that acclimation occurred within the entire year and within both the growing and non-growing seasons and models separated by growing and non-growing seasons better account for seasonal acclimation since R^2 values are usually greater than for those models based on data from the entire year.

Acclimation by loblolly pine throughout the entire year and within seasons has been documented. Teskey *et al.* (1986) reported that loblolly pine seedlings from six provenances had fluctuating rates from August to December when subjected to identical environmental conditions during measurements. Rates peaked in seedlings from all provenances at the end of October and were lowest at the end of September, which coincided with peak leaf growth. The authors concluded that low photosynthesis rates

were the result of high respiration rates due to leaf growth while high photosynthesis rates corresponded to completed leaf growth. Drew and Ledig (1981) determined that seasonal fluctuations in loblolly pine photosynthesis were closely related to progressive shifts in temperature acclimation, reporting that low temperatures limit the northern range of the species. In this study, predicted net photosynthesis was lower during the non-growing season, which could partially be associated with temperature. However, temperature was not a significant variable (relative to PAR and VPD) according to preliminary SAS stepwise models. Possibly, temperatures during measurements were not cold enough to limit photosynthesis since mean monthly temperature was never lower than 12.3°C in the winter (Table 2), which is actually higher than the mid-winter optimum temperature of 10°C proposed by Drew and Ledig.

Seasonal acclimation was observed in the same stand examined in this study at nine-years-old. Murthy et al (1997) found that when photosynthesis was measured under identical environmental conditions (except temperature which was adjusted seasonally) and at saturating light levels, rates were highest in both the control and fertilized stands during the months of March, April, and May and lowest in January, June, July, and September (data was not collected in October, November, or December). The study did not address the effects of water potential on gas exchange however, which may partially explain lower photosynthesis rates in the summer. Data from the study by Murthy *et al.* suggest a trend in which photosynthesis rates reach a lowest maximum rate in September that remains relatively unchanged through the winter.

CHAPTER 6. CONCLUSIONS

Seasonal trends in mean monthly photosynthesis reveal that rates were generally higher, although only significant during April and May, in control stands during the growing season and higher in fertilized stands during the winter. These results suggest that a rapid and large amount of leaf area growth in fertilized stands dilute resources during the growing season, resulting in lower photosynthesis rates. Higher rates in fertilized stands during the winter may be explained by the fact that resources are not allocated to leaf growth and therefore can be used to increase the efficiency of the photosynthetic machinery. Nitrogen content was greater in the fertilized foliage for every month sampled. Results from this study indicate that nitrogen was not diluted during the growing season in fertilized stands and therefore is not responsible for lower rates in fertilized stands. Furthermore, nitrogen was poorly correlated with photosynthesis. Evidence from other studies conducted in the same stands as the current study suggests that the seasonal patterns presented here did not always exist, since fertilized foliage was shown to have higher maximum photosynthesis rates two years after nutrient additions. Logically, photosynthesis must have been greater in fertilized stands at one point to provide the photosynthate necessary for the production of greater leaf areas present in fertilized stands.

Photosynthesis was consistently greater in upper crown foliage compared to the lower crown primarily due to higher light levels in the upper crown, but probably also because of higher photosynthetic capacities in upper crown foliage which are illustrated in predicted light-response curves. Mean monthly conductance and transpiration rates basically mirrored trends in photosynthesis. Mean monthly water use efficiency was generally higher in fertilized stands, but was only significantly greater in March and July, indicating that little difference exists between fertilized and control stands. Significantly greater water use efficiency in the upper crown foliage compared to the lower crown was exhibited during seven months and is attributed mostly to disproportionally higher photosynthesis rates in the upper crown relative to transpiration. Diurnal trends in January and July show that gas exchange was generally not significantly different between fertilization treatments, but gas exchange was consistently greater in the upper crown foliage compared to the lower crown. Gas exchange appeared to fluctuate

throughout the measurement days mostly in response to immediate environmental conditions. January diurnal photosynthesis most closely paralleled light intensity, while July data suggests light intensity and water status influenced photosynthesis since a 40% drop in the afternoon rates corresponded to extremely high VPD values and relatively low water potentials.

Statistical comparisons of both photosynthesis and transpiration model parameter estimates show that common models for all fertilization treatment and crown position combinations, growing and non-growing seasons, and July and January intensives statistically differ enough to justify separate models. Prediction models show that upper crown and lower crown foliage light-response curves generally resemble sun and shade leaf responses, respectively, with upper crown foliage having a higher predicted maximum photosynthesis, respiration rate, light compensation point, and lower predicted initial quantum yield. These responses are most apparent in models for the growing season. Statistical comparisons of parameter estimates confirm several of these differences are significant. Photosynthesis models show that foliage from fertilized stands is more negatively affected by increasing VPD than control foliage, probably because larger leaf areas in fertilized trees result in the need to conserve more water on a per leaf area basis since the transpiration surface area is greater in fertilized trees. Therefore, photosynthesis probably declines due to decreased stomatal conductance in an effort to stabilized or reduce transpiration. However, attempts to model conductance in an effort to confirm stomatal closure in response to VPD were not successful due to significant unexplained variation among rates. This suggests that either conductance was relatively unresponsive to the environment or variability due to other factors (i.e. machine error) was high. Predicted transpiration rates differed highly among all models, however some common trends do exist. During the growing season, rates are predicted to reach a threshold and fall to zero in the lower crown, probably in an attempt to conserve water by only allowing the more photosynthetically active surfaces of the upper crown to transpire. Transpiration is predicted to increase rapidly with increasing VPD during the growing season, possibly because water is not as limiting (as seen in xylem water potentials) or because a regulatory mechanism is not in place.

Evidence from both comparisons of predicted responses to the environment and variability between both the growing season and July diurnal gas exchange models, and the non-growing season and January diurnal models indicates that seasonal acclimation occurred during the course of the this study. Predicted responses to the environment were consistently less variable for the intensive measurement periods compared to the seasonal models, demonstrating that gas exchange is influenced more by the immediate environment on a short-term scale while long-term acclimation may also influence rates over a season.

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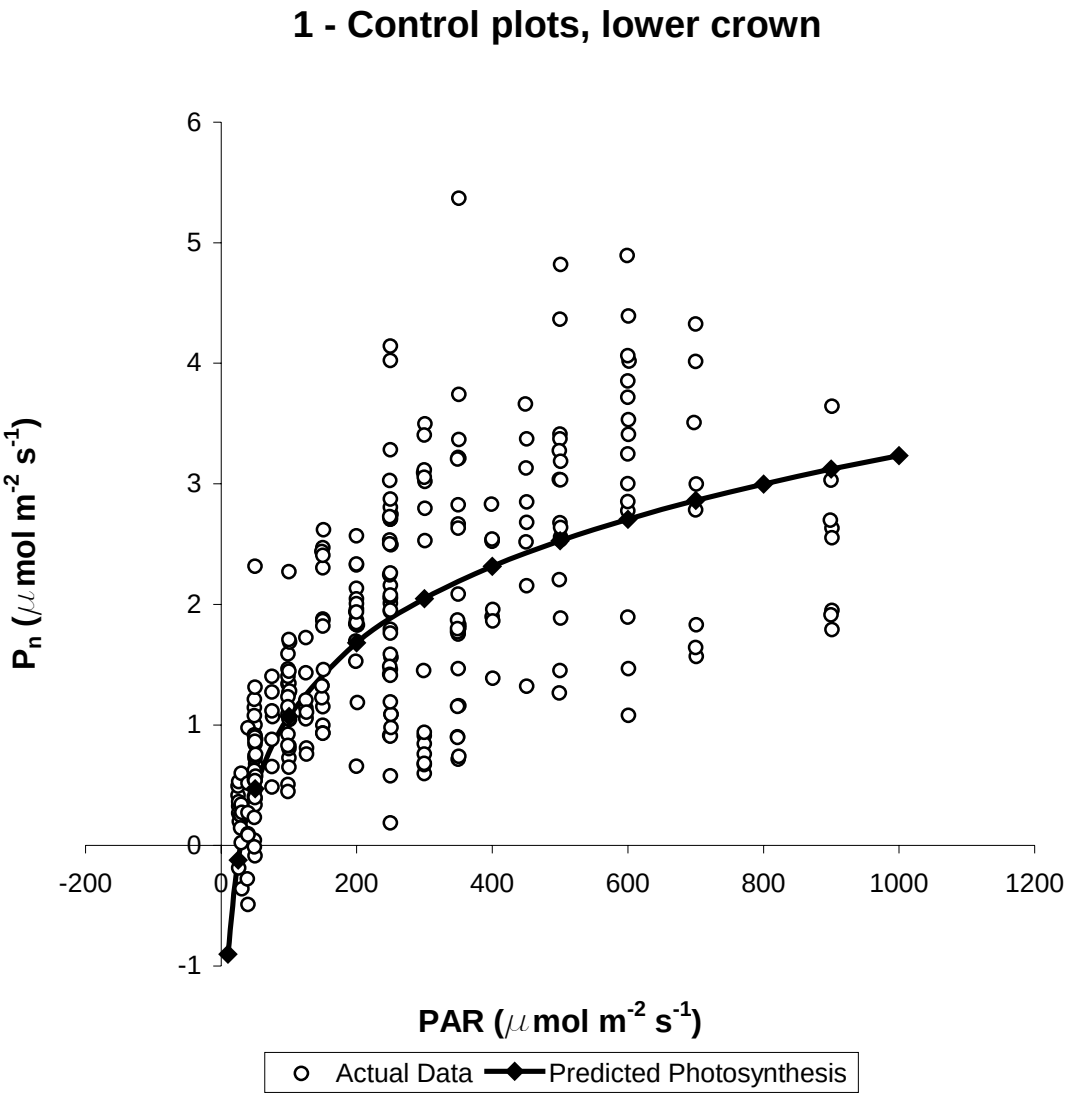
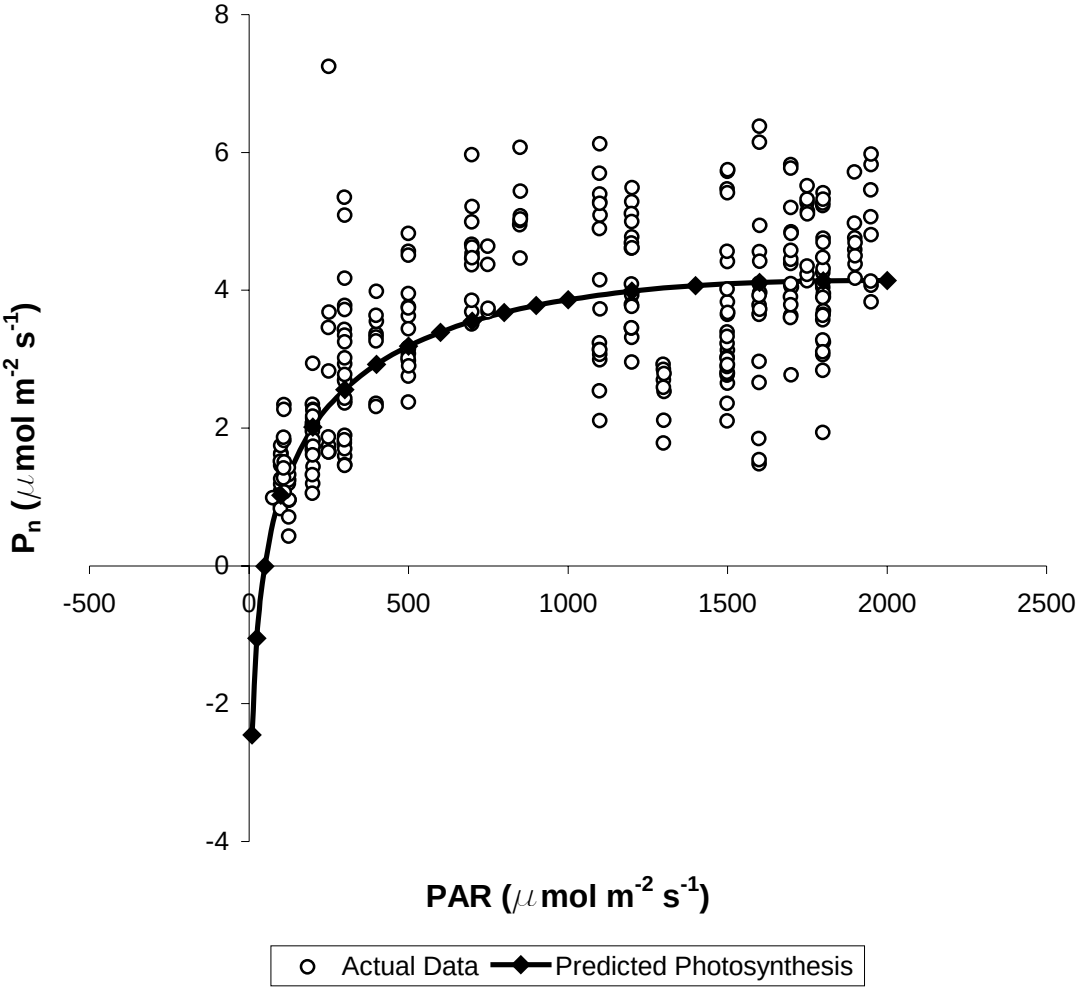
APPENDIX

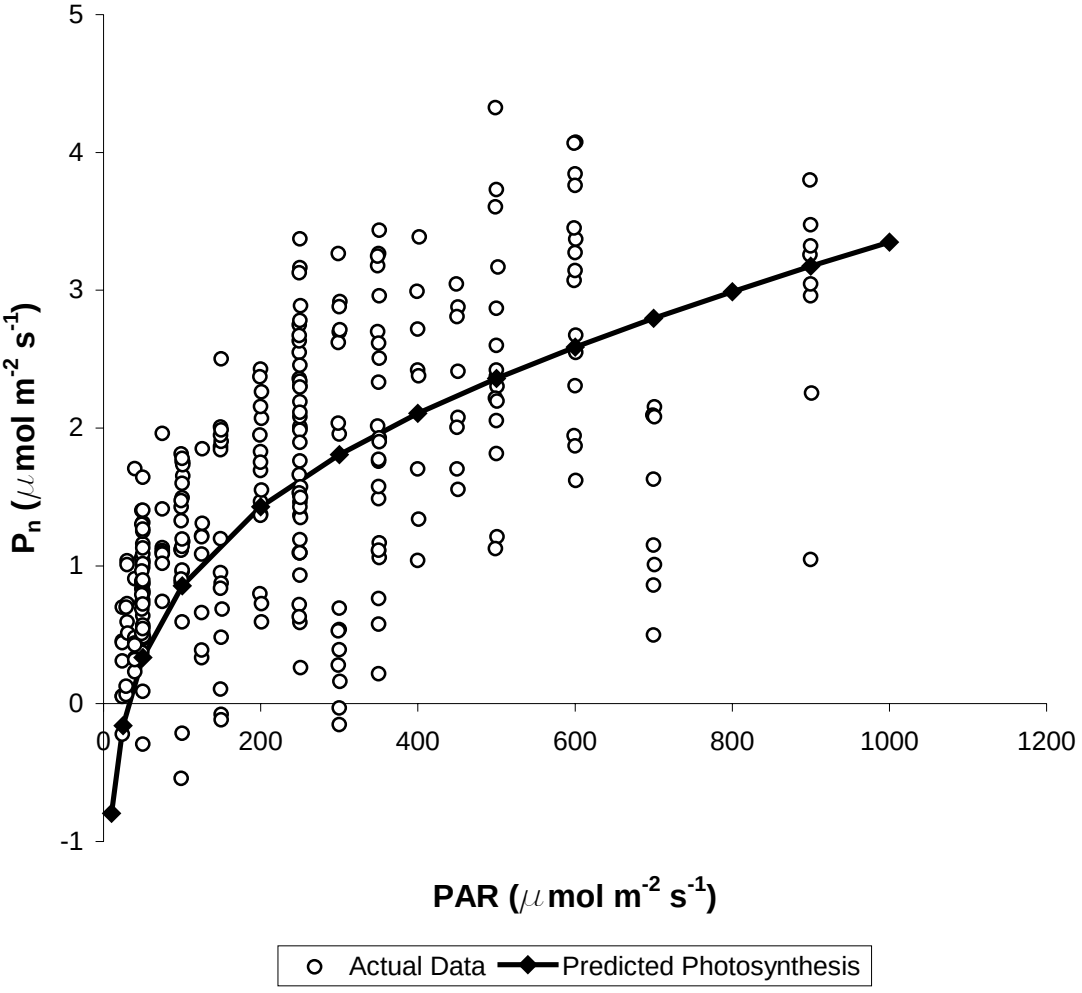


Appendix A: Actual photosynthesis rates for the entire year and predicted photosynthesis rates based on data from entire year plotted against PAR for all fertilization and crown position combinations. Control plots and lower crown (1), control plots and upper crown (2), fertilized plots and lower crown (3), fertilized plots and upper crown (4). Note that axes scales are not necessarily the same. Model parameter estimates and R-square values are listed in Table 3.

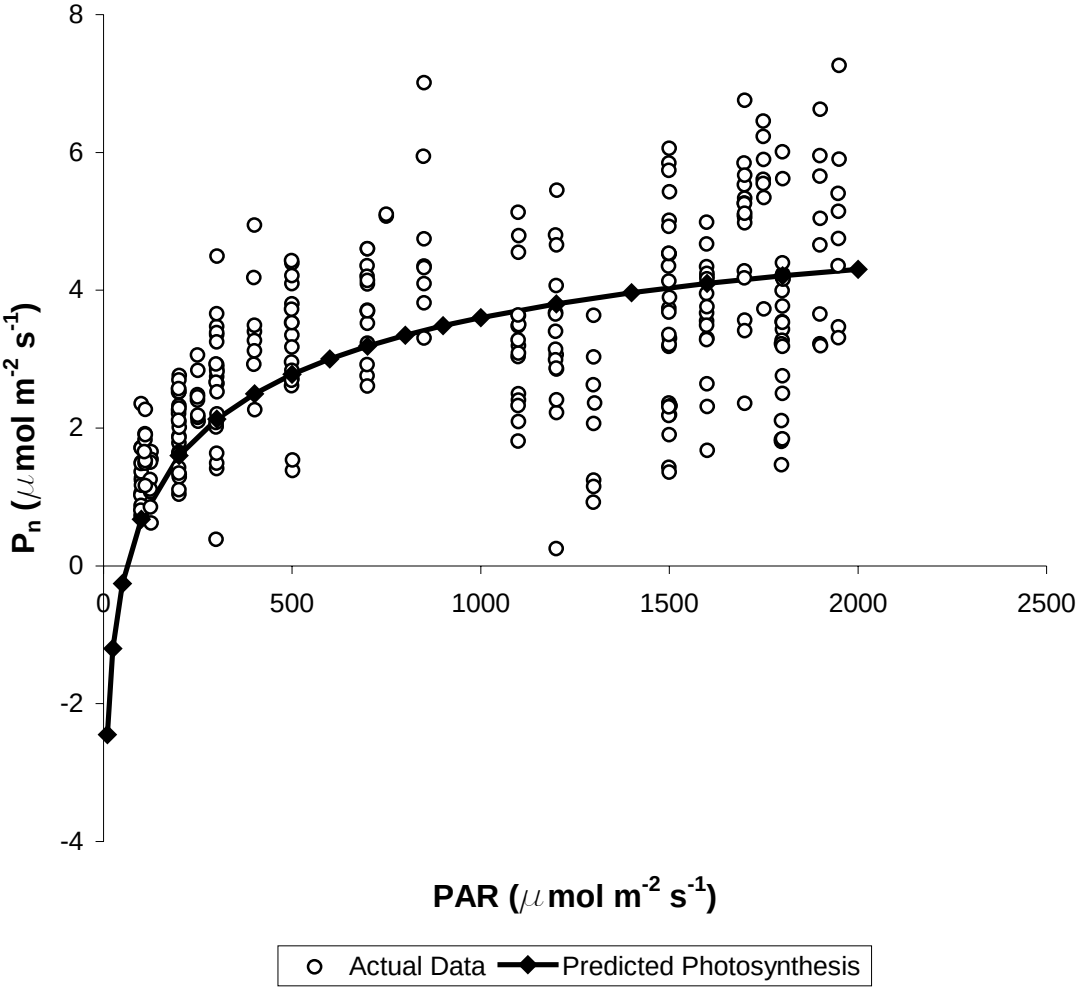
2 - Control plots, upper crown

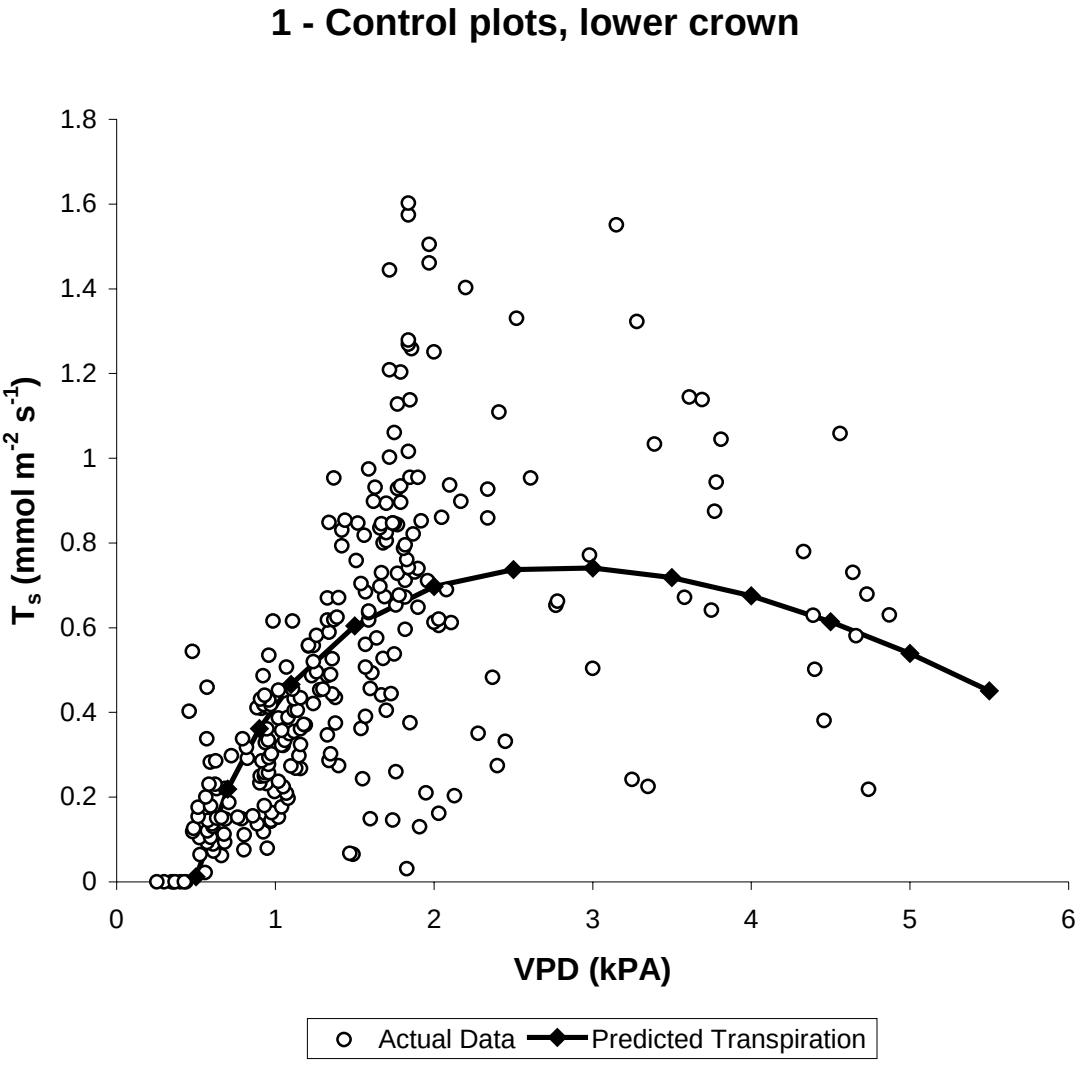


3 - Fertilized plots, lower crown



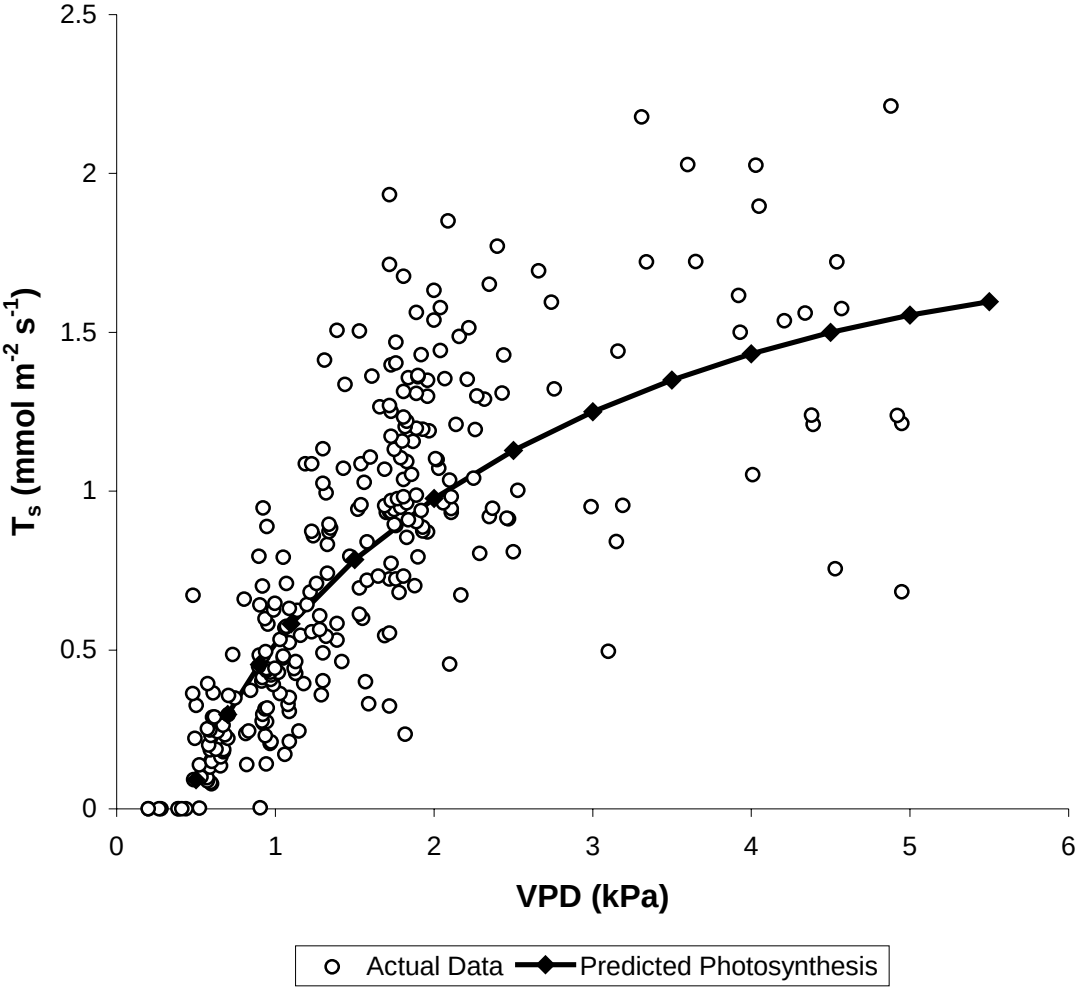
4 - Fertilized plots, upper crown



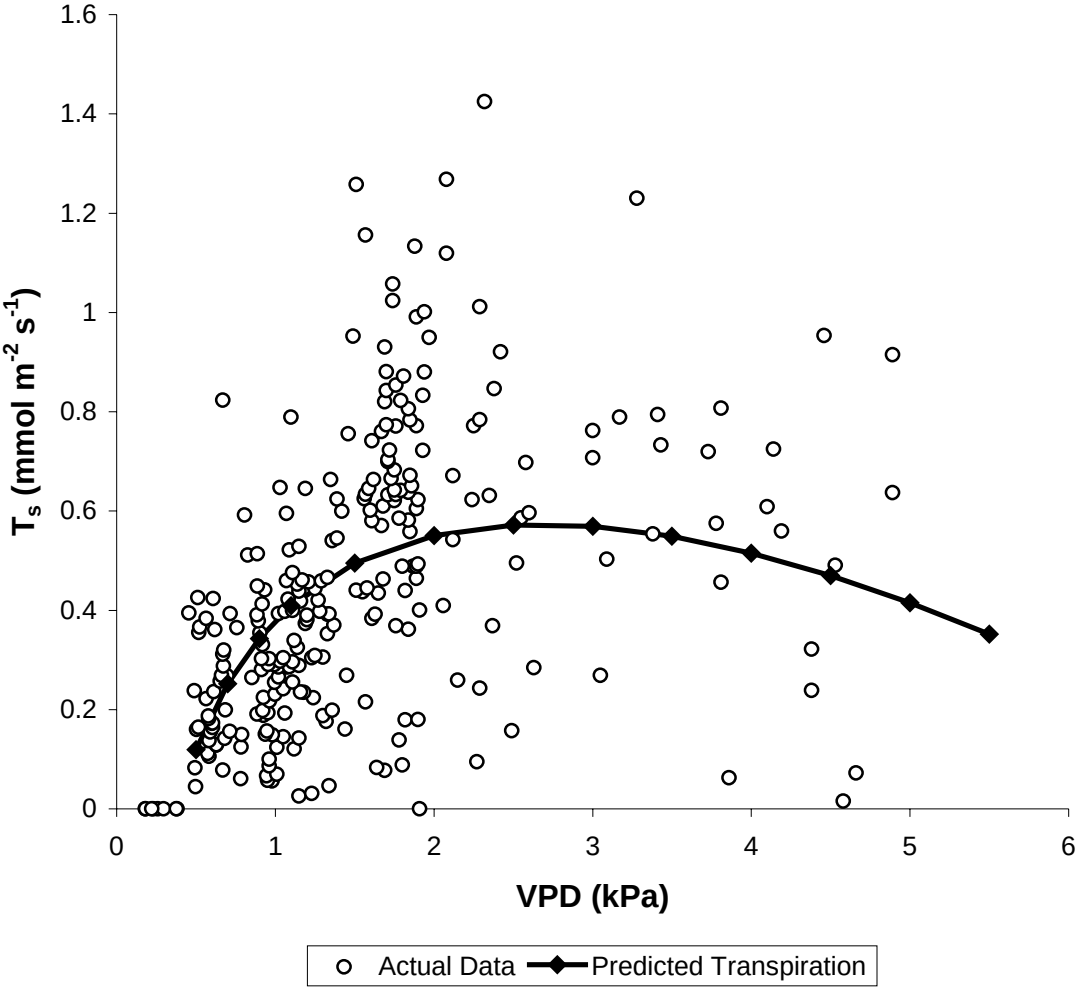


Appendix B: Actual transpiration rates for the entire year and predicted transpiration rates based on data from entire year plotted against VPD for all fertilization and crown position combinations. Control plots and lower crown (1), control plots and upper crown (2), fertilized plots and lower crown (3), fertilized plots and upper crown (4). Note that axes scales are not necessarily the same. Model parameter estimates and R-square values are listed in Table 6.

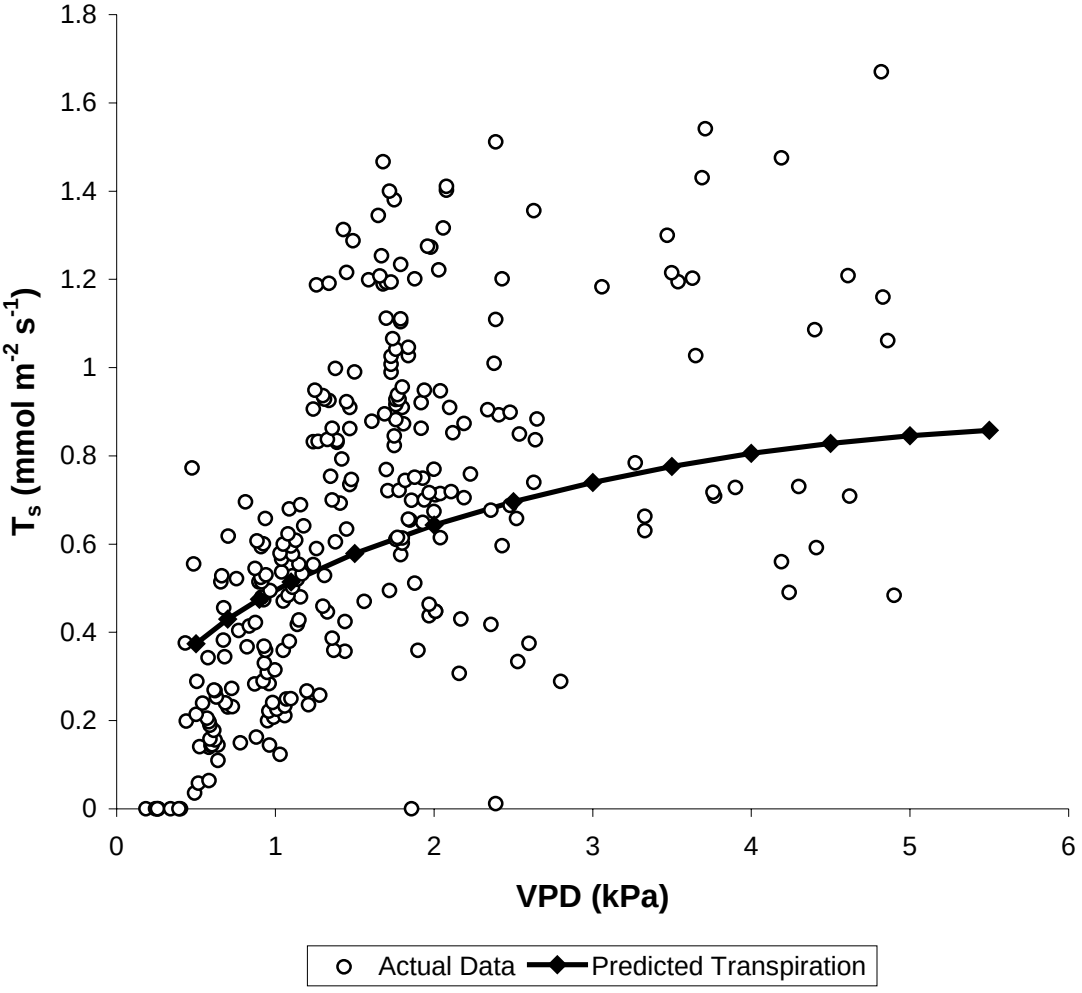
2 - Control plots, upper crown



3 - Fertilized Plots, Lower Crown



4 - Fertilized plots, upper crown



VITA

Chris Gough was born in Madison, Wisconsin in 1975 and attended primary and secondary schools in Oak Ridge, Tennessee and Fredericksburg, Virginia. While earning his B.S. in biology from James Madison University in 1997, Chris wrote an honors thesis based on his undergraduate research titled "The DNA sequence of an *Arabidopsis* α-glucosidase". After receiving his undergraduate degree, Chris worked in the biology department at JMU under Dr. Jon Monroe on a project concerned with the molecular and biochemical characterization of *Arabidopsis thaliana* α-glucosidases. Chris entered the Virginia Tech Department of Forestry as a M.S. candidate in fall of 1998 and will continue in the program as a Ph.D. candidate beginning in fall 2000.