



# Gas exchange and stand-level estimates of water use and gross primary productivity in an experimental pine and switchgrass intercrop forestry system on the Lower Coastal Plain of North Carolina, U.S.A



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## ABSTRACT

Despite growing interest in using switchgrass (*Panicum virgatum* L.) as a biofuel, there are limited data on the physiology of this species and its effect on stand water use and carbon (C) assimilation when grown as a forest intercrop for bioenergy. Therefore, we quantified gas exchange rates of switchgrass within intercropped plots and in pure switchgrass plots during its second growing season in an intensively managed loblolly pine (*Pinus taeda* L.) plantation in North Carolina. Switchgrass physiology was characterized over the growing season from June to October 2010 in terms of photosynthesis ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $\text{mmol m}^{-2} \text{s}^{-1}$ ), and assimilation responses to photosynthetic photon flux density and intercellular carbon dioxide concentration ( $\text{CO}_2$ ). We then used a process-based model of the soil–plant–atmosphere continuum to scale leaf-level gas exchange data to provide estimates of pine and switchgrass stand-level water use (mm) and carbon exchange ( $\text{g C m}^{-2}$ ) over a three-year period. Peak switchgrass photosynthesis ( $32.7 \pm 0.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and stomatal conductance ( $252 \pm 12 \text{mmol m}^{-2} \text{s}^{-1}$ ) rates were measured in July, with minimum values ( $18.7 \pm 1.4 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $104 \pm 6 \text{mmol m}^{-2} \text{s}^{-1}$ , respectively) recorded at the end of the growing season (October). Switchgrass gas exchange and parameter estimates from the light- and  $\text{CO}_2$  response curves did not vary between treatments. However, gas exchange values differed significantly between measurement dates. Model predictions of stand-level transpiration ranged from 287 to 431  $\text{mm year}^{-1}$  for pine and from 245 to 296  $\text{mm year}^{-1}$  for switchgrass. Annual C exchange for loblolly pine ranged from 1165 to 1903  $\text{g m}^{-2}$  compared to 1386 to 1594  $\text{g m}^{-2}$  for switchgrass. At this stage of stand development, no effect of intercropping was evident and there was no effect of distance from the nearest pine row on any switchgrass gas exchange variable measured. However, we anticipate that as this intercropped system develops over time, competition for resources such as light, water or nitrogen may change, with the potential to impact switchgrass physiology and biomass production.

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## 1. Introduction

Economic, environmental and national security concerns associated with using fossil fuels have driven increasing interest in the United States (U.S.) biofuel market and the search for renewable energy sources. Switchgrass (*Panicum virgatum* L.), a perennial,

native warm-season grass, was chosen by the U.S. Department of Energy as the model herbaceous species for development as a biomass energy feedstock (Sanderson et al., 1996; Fuentes and Taliaferro, 2002). Switchgrass has high productivity (Wright, 1994; Downing et al., 1996; Tolbert and Schiller, 1996; McLaughlin et al., 1999), and many positive environmental attributes which include a low nutrient demand (Downing et al., 1996), soil and water conservation benefits (Downing et al., 1996; McLaughlin et al., 1999; George et al., 2008), and increased below-ground C sequestration compared to other herbaceous species (Sanderson et al., 1996;

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Vogel, 2004; McLaughlin and Kszos, 2005). In addition, energy crops such as switchgrass can contribute to the economic viability and crop diversity of landowners (Sanderson et al., 1996; Tolbert and Schiller, 1996).

One approach currently being investigated is intercropping, where switchgrass is grown between rows of planted trees within intensively managed forests. Switchgrass and any tree components not traditionally used as forest products (e.g. thinning and harvest residues) could be used as feedstocks for biofuel production. Benefits of intercropping include a reduction in the fuel versus food conflict, lower risks associated with entering a relatively new biofuel market, increased yields by making optimum use of available growing space (Blazier, 2009), and compatibility with existing biomass handling procedures. Yields are driven by resource availability and use efficiency, and physiological activity, which in turn are governed by environmental and plant factors, and their interactions (Kozlowski and Pallardy, 1997). Environmental factors include photosynthetic photon flux density, air and soil temperature, CO<sub>2</sub> concentration, water, humidity, and mineral nutrients (Kozlowski and Pallardy, 1997; Long, 1999; Moore et al., 2004). Plant factors include stomatal characteristics, leaf age and source-sink relationships (Kozlowski and Pallardy, 1997). Stomatal conductance, in turn, is driven mainly by photosynthetic photon flux density, vapor pressure deficit (Thorpe et al., 1980; Whitehead et al., 1981), temperature, leaf water potential and CO<sub>2</sub> concentration (Jarvis, 1976; Thornley, 1996).

Another advantage of intercropping is the potential to maximize site resource use efficiencies. For example, the inter-row area between trees often contains competing vegetation that is temporarily controlled mechanically or by herbicides, both requiring additional costs (Fox et al., 2007). Intercropped switchgrass could use resources in this area to produce a viable feedstock, providing additional revenue, thereby improving soil water and nutrient use efficiencies. Further, as physiology of trees (C<sub>3</sub> species) and switchgrass (C<sub>4</sub> species) are temporally offset during the growing season (Barnes et al., 1983; Davidson and Csilag, 2003), more water could be available to trees as many species of competing vegetation are C<sub>3</sub> competitors and physiologically active at the same time as crop trees. The C<sub>4</sub> photosynthetic pathway has been well characterized with respect to light, temperature, water, and nitrogen (N) (Black et al., 1969; Downes, 1969; Hatch, 1976; Percy and Ehleringer, 1984; Long, 1999). Loblolly pine physiology has been extensively documented (e.g. Maier et al., 2002; Tyree et al., 2009; Ellsworth et al., 2012). However, there are virtually no data available on switchgrass physiology when grown as a biofuel in an operationally intercropped pine plantation system within a low pH environment, and there are no data available on leaf-level physiology and water relations of switchgrass and loblolly pine when intercropped. King et al. (2013) performed a comparative analysis of water use by representative lignocellulosic bioenergy species and concluded that the data needed to design water-efficient bioenergy-cropping systems are currently not available. In order to design these types of systems, and to improve future model parameterizations, detailed knowledge of the ecophysiology and water relations of the major bioenergy crops under realistic field conditions is crucially needed (King et al., 2013).

A key uncertainty of the ecophysiological performance of pine-switchgrass intercropping systems is that site preparation in forestry is very different from that in agriculture. In forestry, non-merchantable residual logging material from harvesting is normally retained, and site preparation may involve chopping, windrowing, broadcast burning, V-shearing, disking, herbicide use, and/or bedding (Nilsson and Allen, 2003; Fox et al., 2007). In contrast, switchgrass is typically drilled in a conventional seedbed or established by no-till seeding methods (e.g. broadcast). A seedbed of exposed mineral soil is critical, and factors such as proper

planting depth and effective weed control strongly influence switchgrass establishment success (Lewandowski et al., 2003; George et al., 2008). Different levels of site preparation were employed in the current study: some plots were V-sheared prior to planting, whereas others were V-sheared and root raked, thereby creating a more uniform seed bed, but also a higher level of disturbance. Further, coarse woody material from harvesting the previous rotation was either removed, or retained on site. As in forestry, differences in site preparation can result in differences in resource availability, specifically soil N, and therefore physiology and productivity (Tamm, 1964; Li et al., 2003; Kimmins, 2004; Zerpa, 2010), and soil moisture (Entry et al., 1987; Smethurst and Nambiar, 1990; Roberts et al., 2005).

A long-term field study was established on the Lower Coastal Plain of North Carolina, U.S.A., to determine effects of intercropping loblolly pine (*Pinus taeda* L.), grown for solid wood products, with switchgrass grown for biofuel production (Albaugh et al., 2012). This multifunctional intercropped production system has potential to be broadly applicable throughout the southeast U.S. This study provided an opportunity to examine switchgrass physiology in an intercropped system and to provide baseline gas exchange values for switchgrass grown as a biofuel in a forestry setting. These data can serve as a basis for comparison to determine effects of intercropping and harvest residue management which have the potential to alter site resource availability during the timber rotation. In addition, systematic measurements of physiological processes across growing seasons and various environmental conditions are needed as inputs, and for validation, of process-based models (Constable and Friend, 2000; Sands, 2003; Dohleman et al., 2009). One such model is the soil-plant-atmosphere (SPA) model of Williams et al., (1996, 2001a), which has been tested and validated across a diverse range of species and ecosystems (Williams et al., 1998, 2000, 2001b; Fisher et al., 2006; Zeppel et al., 2008; Macinnis-Ng et al., 2011). This process-based model predicts gross primary productivity, canopy water use, stomatal conductance and leaf-level photosynthetic rate.

Our objectives were to (1) measure the seasonal trends in switchgrass leaf-level gas exchange in terms of photosynthesis and stomatal conductance, (2) determine how intercropping in a loblolly pine plantation with forestry site preparation affects switchgrass physiology, (3) quantify CO<sub>2</sub>-assimilation responses to photosynthetic photon flux density and internal CO<sub>2</sub> concentration, and (4) parameterize the SPA model for this specific site for pine and switchgrass, and then use the model to scale leaf-level gas exchange data to predict stand-level water use and C uptake.

## 2. Materials and methods

The long-term field study, termed the Lenoir 1 Intercropping Sustainability Study, was established to determine effects of intercropping and biomass management on site productivity and sustainability within the context of intensive forest management for production of solid wood products and biofuel feedstock. This study was established and is being maintained by Catchlight Energy, LLC (a Chevron|Weyerhaeuser Joint Venture) and Weyerhaeuser Company on forest land owned and managed by Weyerhaeuser Company using standard industry practice for the Southeast U.S.A.

### 2.1. Site description and experimental design

The field site was located in Lenoir County, on the Lower Coastal Plain of North Carolina, U.S.A. (35° 16' N, 77° 28' W). Soils were classified as Pantego (fine, loamy, siliceous, semiactive, thermic Umbric Paleaquults) or Rains (fine, loamy, siliceous, semiactive, thermic

Typic Paleaquults) soil series which are deep, poorly drained, moderately permeable soils. The previous stand was a 109 ha loblolly pine plantation planted in 1974, with a site index of 21.3 m at age 25. The study was installed as a randomized complete block design with four blocks (replications). Treatment plots were 0.8 ha in size with 0.4 ha measurement plots. There was at least 15 m of buffer area between treatment plots. As the objective of our study was to measure leaf-level gas exchange of switchgrass, the following three treatments were selected from three out of the four blocks: switchgrass-only, and two treatments where pine was intercropped with switchgrass. The two treatments where pine was intercropped with switchgrass were laid out in combination with different biomass management options, either: (1) Biomass retained (designated as 'intercropped + biomass'), where all non-merchantable logging material from harvesting the previous rotation remained on site (as is standard practice), or (2) Biomass removed (designated as 'intercropped - biomass'), where any non-merchantable material that could potentially be used for biofuel production (i.e. coarse woody debris >5 cm in diameter) was removed by a grapple-claw excavator and piled along the sides of each treatment plot; remaining woody material was left as groundcover within the plot boundary. See Albaugh et al. (2012) and Leggett and Sucre (2012) for a full description of the study site and respective treatments.

The pine-switchgrass intercropped treatments were V-sheared and bedded with a bulldozer and pine trees were planted by hand during winter 2008 on bedded rows spaced 6 m apart and 1.6 m apart within rows at 1075 stems ha<sup>-1</sup>. Weyerhaeuser's liquid suspension-based fertilizer with 3% N, 6.2% phosphorus (P), 2.5% potassium (K), 4.5% magnesium (Mg) and 2% calcium (Ca) was incorporated into beds to promote seedling root development and establishment. Prior to planting switchgrass, the inter-bed areas between the pine tree rows were V-sheared. In the switchgrass-only treatment, the entire plot area was V-sheared and root raked (thereby removing most harvest residuals).

Coarse woody debris (CWD) mass (>5 cm diameter), which excluded sheared stumps, and corresponding C and N contents removed during site preparation were quantified by Beauvais (2010). There was 10.6 Mg CWD ha<sup>-1</sup> containing 5.1 Mg C ha<sup>-1</sup> and 15.5 kg N ha<sup>-1</sup> in the intercrop + biomass treatment versus 1.7 Mg CWD ha<sup>-1</sup> containing 0.8 Mg C ha<sup>-1</sup> and 2.7 kg N ha<sup>-1</sup> in the intercrop - biomass treatment at the beginning of the trial. Removal of CWD did not affect inorganic soil N or potentially mineralizable N when measured at the beginning of the trial: extractable NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations were 26.0 ± 2.2 kg ha<sup>-1</sup> and 5.0 ± 1.9 kg ha<sup>-1</sup>, respectively, with potentially mineralizable N of 66.8 ± 11.8 kg ha<sup>-1</sup> (see Albaugh et al., 2012).

## 2.2. Switchgrass establishment

Switchgrass (cultivar Alamo) seed was machine-planted in June 2009 at 9 kg pure live seed ha<sup>-1</sup> in rows spaced 40 cm apart. Seed was planted at a depth of 0.6 cm and covered with soil. These seeding rates and planting depths were consistent with recommended values (Wolf and Fiske, 1995; George et al., 2008). Simultaneous with planting, the aforementioned liquid suspension fertilizer was applied between each row of switchgrass. The combination of high P and base cations in this fertilizer was intended to increase soil pH, which was inherently very acidic (pH 3.9), and promote root growth. In the switchgrass-only plots, the entire 0.8 ha plot was planted to switchgrass in rows spaced 40 cm apart. In the intercropped plots, switchgrass seed was planted between each pine row in a swath approximately 2 m wide; there were six 2 m-wide rows of intercropped switchgrass in each 0.4 ha measurement plot. Switchgrass plots were sprayed with 2,4-D (4.68 L ha<sup>-1</sup>) and a post-emergent herbicide (Basagran; 0.88 L ha<sup>-1</sup>) in May 2010.

Weyerhaeuser's coated urea Arborite<sup>®</sup> fertilizer, supplying 65.6 kg N ha<sup>-1</sup>, 6.6 kg P ha<sup>-1</sup> and 0.2 kg boron ha<sup>-1</sup>, was applied in June 2010. Fertilizer levels were determined based on published literature, where typical recommendations of N applications for switchgrass range from 50 to 112 kg ha<sup>-1</sup> (Bredja, 2000; Lemus, 2004; Garland, 2008; Lemus et al., 2009).

## 2.3. Meteorological data

Meteorological data were collected from an automatic weather station located on-site. Sensors recorded temperature (°C) and relative humidity (%) (HOBO Temp/RH Smart Sensor S-THB-M002), photosynthetically active radiation (HOBO PAR Smart Sensor S-LIA-M003), rainfall (HOBO 0.2 mm tipping bucket Rain Gauge Smart Sensor S-RGB-M002), and windspeed (HOBO Windspeed/Direction Smart Sensor S-WCA-M003; Onset Computer Corporation, Bourne, MA, U.S.A.). A HOBO U30 NRC data logger (Onset Computer Corporation, Bourne, MA, U.S.A.) was programmed to log data at 30 min intervals, which were averaged or totaled over a 24 h period each day. Solar radiation (measured using a pyranometer; LI-200S, LI-COR Inc., Lincoln, NE, U.S.A.) and any missing data from the on-site meteorological station were obtained from a nearby weather station (35°18'N and 77°34'W) located at a distance of 10.4 km from the trial site, and with an altitude difference of 10 m.

## 2.4. Soil water and temperature

Volumetric soil water content (VSWC) and temperature data were recorded on a daily basis from July to October 2010. Four soil water and temperature probes (Decagon Devices, Inc., Pullman, WA, U.S.A.) were installed per plot in the intercropped treatments: probes were oriented horizontally at a depth of 10 and 30 cm in the bed and interbed locations. Two probes (one at each depth) were installed in the pure switchgrass plots. All soil water and temperature data presented for the intercropped treatments were calculated from probes in the interbed location only, based on the assumption that switchgrass had not rooted in the beds at this time.

## 2.5. Switchgrass seasonal gas-exchange measurements

Leaf-level gas exchange variables (photosynthesis ( $A_{max}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ )) were measured four times over the 2010 growing season on the youngest, unshaded, fully expanded switchgrass leaf blade from six individual tillers per plot, using an open-path portable photosynthesis system (LI-6400, LI-COR, Inc., Lincoln, NE, U.S.A.) equipped with a 6 cm<sup>2</sup> cuvette. Gas exchange rates were measured between 1000 and 1400 h under predominantly clear skies, with photosynthetic photon flux density (PPFD) and reference CO<sub>2</sub> concentration held constant at 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and 380  $\mu\text{mol mol}^{-1}$ , respectively. All other variables remained at ambient conditions. Cuvette temperatures averaged 33.9 °C in June, 37.5 °C in July, 38.3 °C in August, and 32.2 °C in October. Data were logged when chamber conditions reached equilibrium. Calipers were used to measure the mid-point width of each leaf enclosed in the cuvette, and all gas exchange measurements were recalculated based on leaf area in the cuvette.

Switchgrass gas exchange measurements in the intercrop treatments were based on a stratified random sampling approach. The 2 m-wide planted switchgrass area between each pine row was divided longitudinally into three zones and one measurement was randomly collected from each zone within each of the six rows of intercropped switchgrass ( $N=6$  per plot). At each measurement point, distance (m) to the nearest pine row was recorded to determine whether there was a significant effect of distance from a pine row on any of the switchgrass gas exchange parameters. The same

sampling design was used in the switchgrass-only plots for comparison across all treatments, i.e. in each switchgrass-only plot, six 2 m-wide permanent sampling areas were established; these areas were divided into three longitudinal zones, and two measurements were randomly collected from each zone.

## 2.6. Light-response and A–C<sub>i</sub> curves

We measured CO<sub>2</sub> assimilation responses of switchgrass to photosynthetically active radiation (light-response curves) and internal CO<sub>2</sub> concentrations (A–C<sub>i</sub> curves) on two days in July 2010. Quantum yield parameters, carboxylation efficiency and maximum ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity were estimated from these data to explore physiological mechanisms underlying gas exchange patterns. Light-response curves were measured on 1 and 28 July (Day of Year (DOY) 182 and 209, respectively), and A–C<sub>i</sub> curves were measured on 2 and 29 July (DOY 183 and 210, respectively), on the youngest unshaded fully expanded leaf blade from two individual tillers per plot using a LI-6400 (LI-COR, Inc., Lincoln, NE, U.S.A.). We adopted a similar stratified random sampling approach outlined above for gas exchange measurements; however, one measurement was collected from the middle of the planted switchgrass area (i.e. relatively further away from a pine row), and one from the edge of the planted switchgrass area (i.e. relatively closer to a pine row; N = 2 per plot).

Switchgrass light response curves were measured at a constant CO<sub>2</sub> concentration of 380 μmol mol<sup>-1</sup> and photosynthetic photon flux densities (PPFD) of 0, 200, 400, 600, 800, 1000, 1500 and 2000 μmol m<sup>-2</sup> s<sup>-1</sup>. The relationship between net assimilation and intercellular CO<sub>2</sub> concentration was examined at a constant PPFD of 1600 μmol m<sup>-2</sup> s<sup>-1</sup> and external CO<sub>2</sub> concentrations of 50, 100, 200, 300, 400, 600 and 800 μmol mol<sup>-1</sup>. Chamber conditions were held constant for 2–3 min before all data were logged. Measurements were made between 1000 and 1430 h. Cuvette temperature was maintained at concurrent ambient temperature and averaged 32.7, 32.4, 36.6 and 37.0 °C for DOY 182, 183, 209 and 210, respectively. Leaf area enclosed in the cuvette was calculated as for the gas exchange measurements. The response of A to changes in PPFD was modeled using a non-rectangular hyperbola (Marshall and Biscoe, 1980) to determine the light saturated photosynthetic rate (A<sub>sat</sub>, μmol m<sup>-2</sup> s<sup>-1</sup>), the apparent quantum yield of photosynthesis (α, μmol CO<sub>2</sub> μmol<sup>-1</sup> absorbed quanta), theta (θ, a unit-less parameter which describes the sharpness of the transition from light limitation to light saturation), and the rate of dark respiration (R<sub>d</sub>, μmol m<sup>-2</sup> s<sup>-1</sup>). Parameters calculated from the A–C<sub>i</sub> curves were the carboxylation efficiency (k, mol m<sup>-2</sup> s<sup>-1</sup>), which was determined from the gradient of the slope of the linear portion of the response of A against C<sub>i</sub> (Ku and Edwards, 1977; Collatz et al., 1992), and maximum Rubisco activity (V<sub>cmax</sub>, μmol m<sup>-2</sup> s<sup>-1</sup>), calculated from the C<sub>i</sub>-saturated value of A, after the theory of Collatz et al. (1992).

## 2.7. Soil–plant–atmosphere model

The soil–plant–atmosphere (SPA) model simulates ecosystem photosynthesis, plant water use and stand water balance at fine temporal and spatial scales (30 min time-step, with multiple canopy and soil layers; see Williams et al., 1996, 2001a for a full description). The scale of parameterization (leaf-level) and prediction (canopy-level) of this model were designed for scaling up leaf-level processes to canopy and landscape scales.

The SPA model was parameterized for pine and switchgrass at our site using local meteorological measurements, soil properties and plant characteristics (Table 1). We compared model outputs against measurements of leaf-level photosynthesis, g<sub>s</sub> and water

potential, and then used the model to predict stand-level water use and C exchange over a three-year period (2010–2012). Simulations commenced in January of each year and were terminated for switchgrass in November prior to harvesting in December. Methods used to derive model input and validation values are briefly described in the following sections. Data for model parameterization were collected from pure pine and switchgrass, and intercrop + biomass treatments. These data were collected during 2010, 2011 and 2012 for switchgrass, and 2011 and 2012 for pine.

### 2.7.1. Soil parameters

Soil particle size analysis (% sand, silt and clay) was measured in the top 15 cm of the soil using the hydrometer method (Bouyoucos, 1962; Gee and Bauder, 1986), based on one soil sample collected per plot. Volumetric soil water content at 10 and 30 cm depths was recorded at the start of the study period (see *Soil water and temperature* section above).

### 2.7.2. Atmospheric variables

Atmospheric input variables were collected as part of routine weather data measurements and are described above in the *Meteorological data* section.

### 2.7.3. Plant parameters

In addition to the physiological measurements detailed in earlier sections (see *Seasonal gas-exchange measurements*), the following data were collected and used for SPA model parameterization: leaf and needle width (mm), foliar N concentration (g m<sup>-2</sup>), leaf area index (m<sup>2</sup> m<sup>-2</sup>), leaf water potential (MPa), leaf-level transpiration rates (mmol m<sup>-2</sup> s<sup>-1</sup>), and whole-plant hydraulic conductance (mmol m<sup>-2</sup> s<sup>-1</sup> MPa<sup>-1</sup>).

In 2010, switchgrass leaf width (mm) was measured with a ruler at mid-point on three of the most recent fully-formed leaves per plot on a monthly basis from May to August 2010. In June and October 2011, switchgrass leaf width was measured on all leaves from 18 tillers per plot, and 2012 leaf width was determined in September on 12 of the most recent fully-formed leaves per plot. Pine needle width (mm) was measured with a hand-held ocular lens on individual needles from two fascicles collected from the upper third of the crown from three trees per plot across two blocks in June and October 2011 and April 2012, and from three trees per plot across three blocks in September and October 2012.

Foliar N concentration was determined from aboveground switchgrass biomass collected from three 0.25 m<sup>2</sup> quadrats per plot in May, June, July and September 2010, three 1-m<sup>2</sup> quadrats per plot in June and October 2011, and three 3-m<sup>2</sup> quadrats in September 2012. Pine foliar N concentration was determined from 10 fascicles collected per tree from eight trees per plot across two blocks in July, September and November 2011. Individual samples per species were combined by plot, ground to pass through a 2.0-mm screen, and N concentration was analyzed by combustion using a LECO TruSpec CHN analyzer (LECO Corporation, St. Joseph, MI, U.S.A.).

Switchgrass LAI values for 2010 were obtained from Albaugh et al. (2012). In 2011 and 2012, switchgrass LAI was determined from biomass collected from three 1-m<sup>2</sup> quadrats per plot in June and October 2011, and three 3-m<sup>2</sup> quadrats in September 2012. Approximately six tillers were randomly selected from each quadrat, height (cm) was measured from the soil surface to the collar of the tallest leaf blade, and then separated into leaf blades and stalks. The remaining biomass was dried at 65 °C to a constant weight (g). Leaf area of the six selected tillers was determined by tracing each leaf blade, cutting these out by hand and scanning using an Epson scanner (Epson America, Inc., Long Beach, CA, U.S.A.). The subsequent digital image was analyzed for leaf area with ImageJ software [<http://rsb.info.nih.gov/ij/docs/>]. Stalk area (cm<sup>2</sup>) was calculated from the area of a cylinder (2 pi × radius × height). Leaf and

**Table 1**

Parameter values and variables used in the soil–plant–atmosphere model to provide stand-level water use and carbon exchange estimates for switchgrass and loblolly pine grown in a forested setting on the Lower Coastal Plain of North Carolina, U.S.A. from 2010 to 2012.

Parameter/variable	Units	Value		Source <sup>a</sup>
Atmospheric variables (half-hourly range over 2010–2012)				
Air temperature	°C	–11.5 to 39.5		Measured, this study
Dewpoint temperature	°C	–17.8 to 26.8		Measured, this study
Relative humidity	%	0–100		Measured, this study
Wind speed	km h <sup>-1</sup>	0.0–53.4		Measured, this study
Rainfall	mm	0.0–40.4		Measured, this study
Solar radiation	W m <sup>-2</sup>	0–651		Measured, this study
Photosynthetically active radiation	μmol m <sup>-2</sup> s <sup>-1</sup>	0–2554		Measured, this study
Vapor pressure deficit	kPa	0.0–3.5		Measured, this study
Parameter/variable	Units	Value		Source <sup>a</sup>
		Switchgrass	Pine	
Soil parameters				
Sand content in top 15 cm	%	68	64	Measured, this study
Clay content in top 15 cm	%	27	21	Measured, this study
Silt content in top 15 cm	%	5	14	Measured, this study
Volumetric soil water content (half-hourly range over 2010 to 2012, averaged over 10 and 30 cm depths)	m <sup>3</sup> m <sup>-3</sup>	0.178–0.434	0.061–0.419	Measured, this study
Parameter/variable	Units	Value		Source <sup>a</sup>
		Switchgrass	Pine	
Plant parameters				
Leaf area index	m <sup>2</sup> m <sup>-2</sup>	0.0–3.3	0.3–1.9	Albaugh et al. (2012); Measured, this study
Belowground biomass	g m <sup>-2</sup>	399–518	320–417	Calculated as a ratio of aboveground biomass (see Albaugh et al., 2012), based on Garten et al. (2010) (SG); Unpublished data from nearby 6-year-old loblolly pine site (P). Wilson (1998), Gill and Jackson (2000), Gill et al. (2002) (SG); King et al. (2002) (P)
Fine root turnover	%	60	119	Wilson (1998), Gill and Jackson (2000), Gill et al. (2002) (SG); King et al. (2002) (P)
Rooting depth	m	0.6	0.6	Calculated using a logistic dose-response curve (Schenk and Jackson, 2002)
Foliar N concentration	g m <sup>-2</sup>	4.4	2.3	Measured, this study
Leaf width	mm	10	1.8	Measured, this study
Leaf-level photosynthesis	μmol m <sup>-2</sup> s <sup>-1</sup>	5.6–34.4	2.1–6.9	Measured, model output <sup>d</sup>
Leaf-level stomatal conductance	mmol m <sup>-2</sup> s <sup>-1</sup>	25–275	36–124	Measured, model output <sup>d</sup>
Leaf water potential	MPa	–0.08 to –1.68	–0.20 to –1.88	Measured, model output <sup>d</sup>
Leaf-level transpiration rate	mmol m <sup>-2</sup> s <sup>-1</sup>	0.2–5.4	0.5–4.9	Measured, this study
Minimum sustainable leaf water potential	MPa	–1.68	–1.88	Measured, this study
Whole-plant hydraulic conductance	mmol m <sup>-2</sup> s <sup>-1</sup> MPa <sup>-1</sup>	3.7	1.3	Measured, this study
Part of whole-plant hydraulic conductance allocated belowground	%	50	50	Sperry et al. (1998), Domec et al. (2009)
V <sub>cm<sub>max</sub></sub> <sup>b</sup>	μmol m <sup>-2</sup> s <sup>-1</sup>	26	51	Measured, this study (SG); Maier et al. (2002); Tyree et al. (2009), Ellsworth et al. (2012) (P)
V <sub>pm<sub>max</sub></sub> <sup>c</sup>	μmol m <sup>-2</sup> s <sup>-1</sup>	52	N/A	Calculated as V <sub>cm<sub>max</sub></sub> *2 (von Caemmerer, 2000)
Maximum electron transport rate	μmol m <sup>-2</sup> s <sup>-1</sup>	145	95	Calculated as V <sub>cm<sub>max</sub></sub> *5.5 (Kim et al., 2006; Massad et al., 2007; Dohleman et al., 2009) (SG); Maier et al. (2002), Tyree et al. (2009), Ellsworth et al. (2012) (P)
Bundle-sheath conductance to CO <sub>2</sub>	mmol m <sup>-2</sup> s <sup>-1</sup>	3	N/A	von Caemmerer (2000)

<sup>a</sup> Values sourced from the literature and specific to a species are indicated for switchgrass (SG) or pine (P).

<sup>b</sup> Rubisco carboxylation capacity.

<sup>c</sup> Maximum phosphoenolpyruvate carboxylase activity.

<sup>d</sup> Data used for validation purposes. N/A = not applicable.

stalk samples used for area determination were dried at 65 °C to a constant weight to calculate specific leaf area (SLA), which was then used to calculate the area of each quadrat based on the dry mass and leaf: stalk ratio. Daily LAI for each year was calculated based on the developmental pattern reported by [Albaugh et al. \(2012\)](#) for this site.

Loblolly pine LAI was determined from phenological measurements conducted in May, July, September, November 2011, and March and August 2012. On each measurement date, tree height (m), diameter at breast height (DBH, 1.3 m above the ground; cm), and height to live crown (m) was measured on seven trees per plot in pure pine and intercrop + biomass plots across two blocks. Branch basal diameter (measured 2 cm from the stem insertion point with caliper jaws parallel to the stem) and distance from groundline was measured for each live branch on each tree. Eight representative branches (covering the range in branch basal diameter and distance from groundline) were collected per plot. Ten fascicles were removed from each branch and placed on ice for transport to the laboratory to determine SLA. Remaining fascicles were dried at 65 °C to a constant weight. Projected area of the ten fascicles was determined by scanning. After scanning, needles were dried at 65 °C to a constant weight (g), SLA was calculated as the ratio of fascicle area to dry mass, and this value was applied to the dry mass of the remaining fascicles to determine needle area. These data were applied to seasonal dynamics of loblolly pine leaf area development ([Blinn et al., 2012](#)) to calculate daily LAI.

Leaf-level photosynthesis and stomatal conductance, and leaf water potential were used to validate the SPA model. Diurnal leaf water potential was measured with a Scholander-type pressure chamber (PMS Instrument Company, Albany, OR, U.S.A.) on three of the most recent fully-formed switchgrass leaves and one pine fascicle collected from the upper third of the crown from three trees per plot from pure pine, pure switchgrass and intercropped + biomass plots in two blocks in June and October 2011 and April 2012, and from three blocks in September and October 2012.

Measurements commenced at predawn and were conducted at regular intervals (approximately every two hours) throughout the day. Leaf-level photosynthesis, stomatal conductance and transpiration rates were measured under ambient conditions on the most recent fully expanded leaf blade from three individual tillers and two pine fascicles collected from the upper third of the crown from three trees per plot using a LI-6400 (LI-COR, Inc., Lincoln, NE, U.S.A.) at regular intervals throughout the day (times corresponded to diurnal leaf water potential measurements except for predawn values).

In the model, maximum flux rate of water through vegetation is determined by the difference between soil and leaf water potential and is controlled by whole-plant hydraulic conductance. Whole-plant hydraulic conductance was calculated from the slope of the curve of transpiration versus leaf water potential ([Loustau et al., 1998](#); [Sperry et al., 2002](#)). The root component was assumed to comprise 50% of plant conductance ([Sperry et al., 1998](#); [Williams et al., 2001a](#); [Domec et al., 2009](#)). In the SPA model, leaf-to-air energy, water and CO<sub>2</sub> exchange consists of a stomatal conductance model coupled with a photosynthesis model ([Williams et al., 1996](#)). As this photosynthesis model is based on C assimilation in C<sub>3</sub> plants (see [Farquhar and Von Caemmerer, 1982](#)), we used it in its original form for pine simulations, but customized the SPA model for use with switchgrass, a C<sub>4</sub> species. The SPA model has been used successfully with C<sub>4</sub> plants (see [Whitley et al., 2011](#)). The C<sub>4</sub> biochemical model we used was based on the theory of [Collatz et al. \(1992\)](#), [von Caemmerer \(2000\)](#) and [Massad et al. \(2007\)](#). Photosynthetic parameters used in the model were taken from von Caemmerer's (2000) Table 4.1, with site-specific parameters listed in our [Table 1](#). Allometric (leaf area index and plant size), hydraulic

and leaf nitrogen content data used to drive the model were measured directly as described above (see [Table 1](#)).

## 2.8. Statistical analyses

We used repeated measures analysis of variance (ANOVA) to test the null hypothesis that switchgrass gas exchange during the 2010 growing season did not differ among treatments using photosynthesis and stomatal conductance as response variables, treatment, day of year and treatment × day of year interactions as fixed effects, block as a random effect, and day of year as the repeated measure. We used a mixed model ANOVA (Proc Mixed, [SAS Institute, 2002](#)) to examine these gas exchange data over the growing season using a heterogeneous autoregressive model of the variance/covariance matrix structure. The same mixed model repeated measures ANOVA was used to test the null hypothesis that VSWC and temperature did not differ between treatments. As VSWC and temperature did not vary significantly with treatment, these data were pooled, and comparisons were presented over the growing season. We used the same basic mixed model repeated measures ANOVA to test the null hypothesis that there was no effect of distance from the nearest pine row on switchgrass gas exchange parameters in the intercropped treatments. In this case, distance from the nearest pine row and its interaction with treatment and day of year were added as fixed effects.

Switchgrass light-response curves were fitted using Proc NLIN, and the carboxylation efficiency was determined from the initial slope of the A-C<sub>i</sub> curves using Proc GLM ([SAS Institute, 2002](#)).

We used an ANOVA (Proc GLM) to test the null hypothesis that parameter estimates from the response curves would not differ by measurement date. If there was no significant difference between the two measurement dates within a treatment, parameter estimates were pooled across dates and comparisons made between the three treatments. Analyses of variance (Proc GLM) were performed to test the null hypotheses that (i) there was no effect of treatment on the parameter estimates of the light- and A-C<sub>i</sub> response curves and (ii) there was no effect of distance from the nearest pine row on switchgrass response curve parameters in the intercropped treatments.

We used a repeated measures ANOVA to test the null hypothesis that diurnal measurements of gas exchange (photosynthesis, stomatal conductance and transpiration) and leaf water potential, used for SPA model parameterization, did not differ between treatments. The same mixed model was used as per the 2010 seasonal gas exchange data, except time of day was used as the repeated measure for each measurement date. Analyses of variance (Proc GLM) were performed to test the null hypothesis that there was no treatment effect on foliar nitrogen concentration, leaf or needle width, or whole-plant hydraulic conductance. In all cases, an  $\alpha = 0.05$  significance level was used. When effects were significant in the ANOVA, least square means were compared using the Tukey-Kramer adjustment method for multiple comparisons between treatments. Dependent variables were checked for normality and homoscedasticity.

Model performance was evaluated using the following outputs from a linear regression (Proc REG) between measured and simulated gas exchange and water potential data: coefficient of determination ( $R^2$ ), the intercept and slope of the regression, and root mean square error.

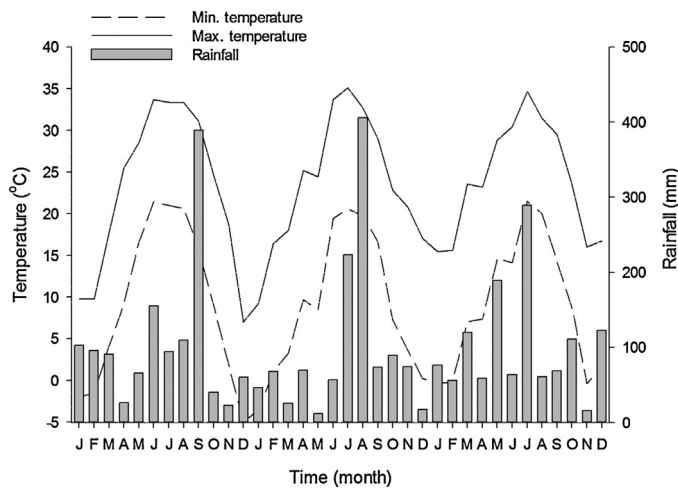
## 3. Results

Data on loblolly pine physiology has been well represented in the literature (see, for example [Maier et al., 2002](#); [Tyree et al., 2009](#); [Ellsworth et al., 2012](#)). Therefore, we focused on presenting

**Table 2**

Differences of the least square means for pairwise day of year (DOY) comparisons for volumetric soil water content (VSWC) and soil temperature, measured at depths of 10 and 30 cm (VSWC\_10, Temp\_10 and VSWC\_30 and Temp\_30, respectively) from July to October 2010 (DOY 195–279). The Tukey-Kramer adjustment method was used to compute *P*-values which are presented for data pooled across pure and intercropped switchgrass treatments.

DOY comparison		VSWC_10	VSWC_30	Temp_10	Temp_30
195	230	0.001	0.029	0.088	0.994
195	279	<.001	<.001	<.001	<.001
230	279	<.001	<.001	<.001	<.001



**Fig. 1.** Average monthly minimum (Min.) and maximum (Max.) temperature and rainfall data measured at the field site on the Lower Coastal Plain of North Carolina from 2010 to 2012. The line graphs represent the temperature, and bars represent the rainfall data.

switchgrass physiology and only present pine data (i) necessary to characterize the site, (ii) used for input into the SPA model (see Table 1), and (iii) used to validate model output.

**3.1. Meteorological data**

Historical (1966–2012) average annual precipitation at this site is 1262 mm and average annual daily temperature is 16.5 °C. Instantaneous minimum and maximum temperatures of –6.0, –11.1, –11.5 °C, and 39.5, 39.4 and 39.5 °C were recorded in January and July of each year (2010, 2011, and 2012), respectively. Mean temperature measured during the growing season (1 April to 30 September) was 24.2, 22.9 and 22.5 °C in 2010, 2011 and 2012, respectively (Fig. 1). Annual precipitation was 1252 mm (2010), 1158 mm (2011) and 1232 mm (2012). Precipitation measured over the growing season was 840 mm (2010), 836 mm (2011) and 731 mm (2012). Minimum monthly rainfall was 26 mm in April

**Table 4**

Differences of the least square means for pairwise comparisons between day of year (DOY) and DOY\*treatment for switchgrass leaf-level photosynthesis. The Tukey-Kramer adjustment method was used to compute *P*-values presented for pure switchgrass, switchgrass intercropped with loblolly pine where material from harvesting the previous rotation was retained on site (intercrop + biomass), and where this harvesting material was removed (intercrop – biomass) for photosynthesis measured over the 2010 growing season from June to October (DOY 160–279, respectively).

DOY comparison		Switchgrass	Intercrop + biomass	Intercrop – biomass
160	195	<.001	<.001	<.001
160	230	0.542	0.295	0.970
160	279	0.843	0.994	1.000
195	230	0.758	1.000	0.012
195	279	<.001	0.004	0.013
230	279	0.018	0.025	1.000

DOY*Treatment comparison		DOY 160	DOY 195	DOY 230	DOY 279
Switchgrass	Intercrop + biomass	1.000	0.984	1.000	1.000
Switchgrass	Intercrop – biomass	1.000	0.971	0.522	1.000
Intercrop + biomass	Intercrop – biomass	1.000	1.000	0.394	1.000

**Table 3**

Differences of the least square means for pairwise day of year (DOY) comparisons for switchgrass gas exchange values measured over the 2010 growing season from June to October (DOY 160–279, respectively). The Tukey-Kramer adjustment method was used to compute *P*-values presented for maximum leaf-level photosynthesis (*A*<sub>max</sub>) and stomatal conductance (*g*<sub>s</sub>) data pooled across treatments.

DOY comparison		<i>A</i> <sub>max</sub>	<i>g</i> <sub>s</sub>
160	195	<.001	<.001
160	230	0.254	0.649
160	279	0.203	0.003
195	230	0.003	<.001
195	279	<.001	<.001
230	279	0.004	<.001

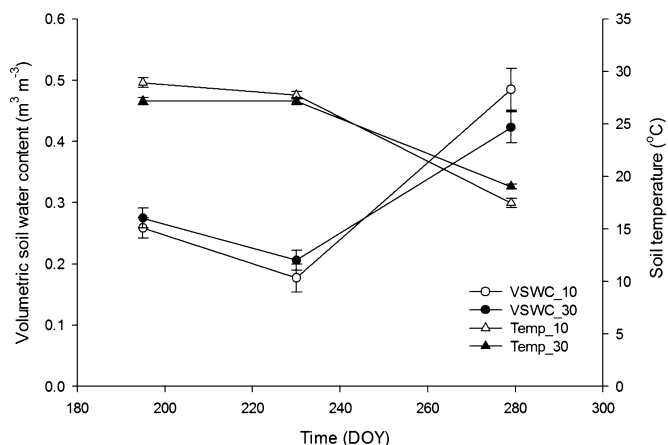
2010, 12 mm in May 2011 and 59 mm in April 2012. Maximum monthly rainfall over the three-year period occurred in September 2010 (389 mm compared to the long-term September average of 127 mm), August 2011 (406 mm compared to the long-term August average of 146 mm) and July 2012 (289 mm compared to the long-term July average of 165 mm).

**3.2. Volumetric soil water content and temperature**

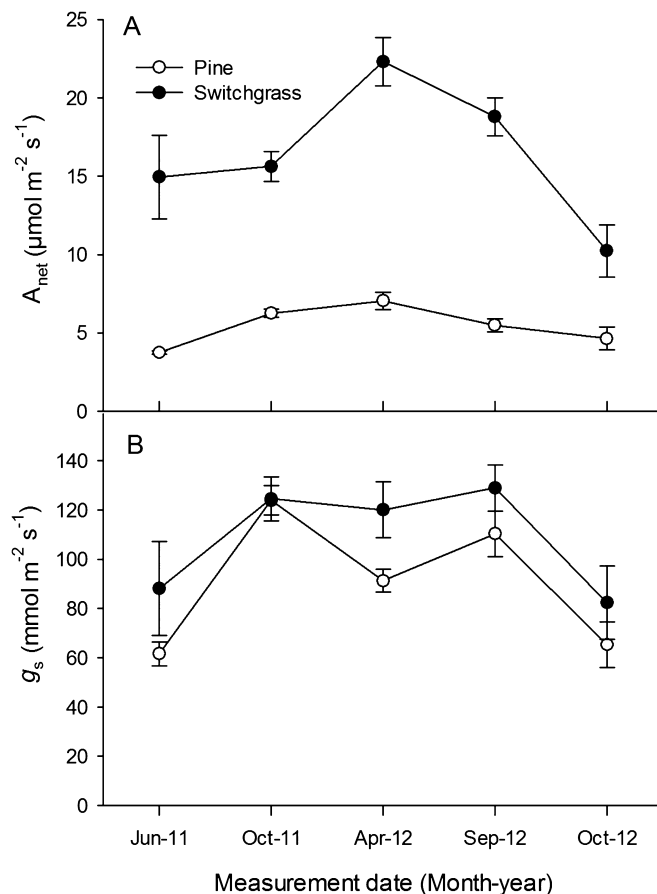
Volumetric soil water content and soil temperature data were pooled across treatments and pairwise DOY comparisons were made (Table 2). Minimum VSWC (0.206 m<sup>3</sup> m<sup>-3</sup>) was measured on DOY 230 (August), compared to a maximum value of 0.423 m<sup>3</sup> m<sup>-3</sup> on DOY 279 (October), driven by high rainfall in September (Fig. 1 and 2). VSWC varied significantly between each measurement date (Table 2). Soil temperature decreased from a maximum value of 27.8 °C on DOY 195 and 230 (July and August, respectively) to a significantly lower temperature of 18.3 °C on DOY 279 (Table 2; Fig. 2).

**3.3. Seasonal gas exchange**

Switchgrass gas exchange did not vary between treatments. However, there were significant (*p* < 0.001) DOY effects and a significant (*p* = 0.037) treatment by DOY interaction for photosynthesis (Tables 3 and 4; Fig. 3). Photosynthesis increased significantly (*p* < 0.001) from 22.0 ± 0.7 μmol m<sup>-2</sup> s<sup>-1</sup> on DOY 160



**Fig. 2.** Volumetric soil water content and soil temperature, measured at depths of 10 and 30 cm (VSWC\_10, Temp\_10 and VSWC\_30, Temp\_30, respectively) from July to October 2010 (day of year (DOY) 195–279). Values presented are means from the switchgrass-only and pine-switchgrass intercropped treatments; error bars indicate the standard error of the mean.



**Fig. 4.** (A) Pine and switchgrass leaf-level assimilation rates ( $A_{net}$ ) and (B) stomatal conductance ( $g_s$ ) measured under ambient field conditions between 1000 and 1400 h from June 2011 to October 2012, and used to validate SPA model outputs. Values presented are means ( $N$  ranged from 12–18); error bars indicate the standard error of the mean.

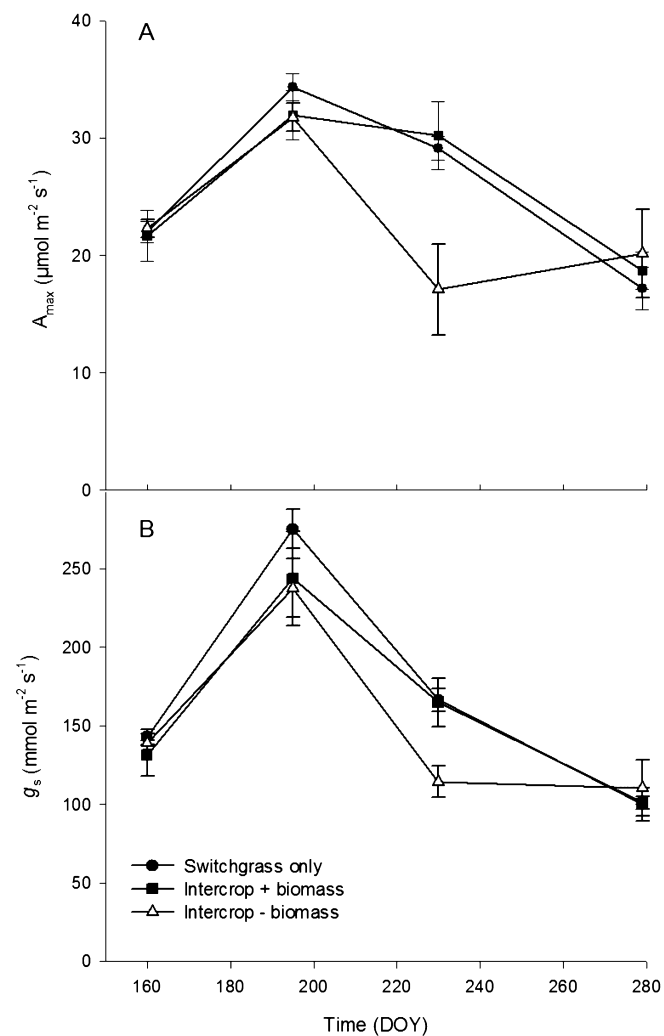
(June) to maximum seasonal values of  $32.7 \pm 0.9 \mu\text{mol m}^{-2} \text{s}^{-1}$  in July (DOY 195), then decreased significantly ( $p < 0.001$ ) through the remainder of the growing season to minimum rates of  $18.7 \pm 1.4 \mu\text{mol m}^{-2} \text{s}^{-1}$  in October (DOY 279) (Fig. 3). Photosynthetic rates in the intercropped-biomass treatment decreased significantly ( $p = 0.012$ ) from July to August (DOY 195–230) (Table 4). Stomatal conductance showed a similar seasonal pattern, reaching peak values in July ( $252 \pm 12 \text{mmol m}^{-2} \text{s}^{-1}$ ; DOY 195), and seasonal lows of  $104 \pm 6 \text{mmol m}^{-2} \text{s}^{-1}$  in October (DOY 279; Fig. 3).

### 3.4. Light and $A-C_i$ response curves

Significance tests of the parameter estimates of light- and  $A-C_i$  switchgrass response curves indicated there were no treatment or DOY differences for  $A_{sat}$ ,  $\alpha$ ,  $\theta$ ,  $R_d$ ,  $k$  or  $V_{cmax}$  (Table 5). Model estimates using all data for light-response curves resulted in an estimated  $A_{sat} = 28.7 \pm 2.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $\alpha = 0.059 \pm 0.012 \mu\text{mol CO}_2 \mu\text{mol}^{-1}$  absorbed quanta,  $\theta = 0.74 \pm 0.18$  and  $R_d = 3.4 \pm 0.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Estimates of  $k$  and  $V_{cmax}$  using all data from the  $A-C_i$  curves were  $0.31 \pm 0.02 \text{mol m}^{-2} \text{s}^{-1}$ , and  $27.6 \pm 1.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively.

### 3.5. Effect of proximity to a pine row on gas exchange parameters

There was no significant effect of distance from the nearest pine row on switchgrass gas exchange parameters in the intercropped treatments ( $p = 0.246$  and  $0.152$  for photosynthesis and



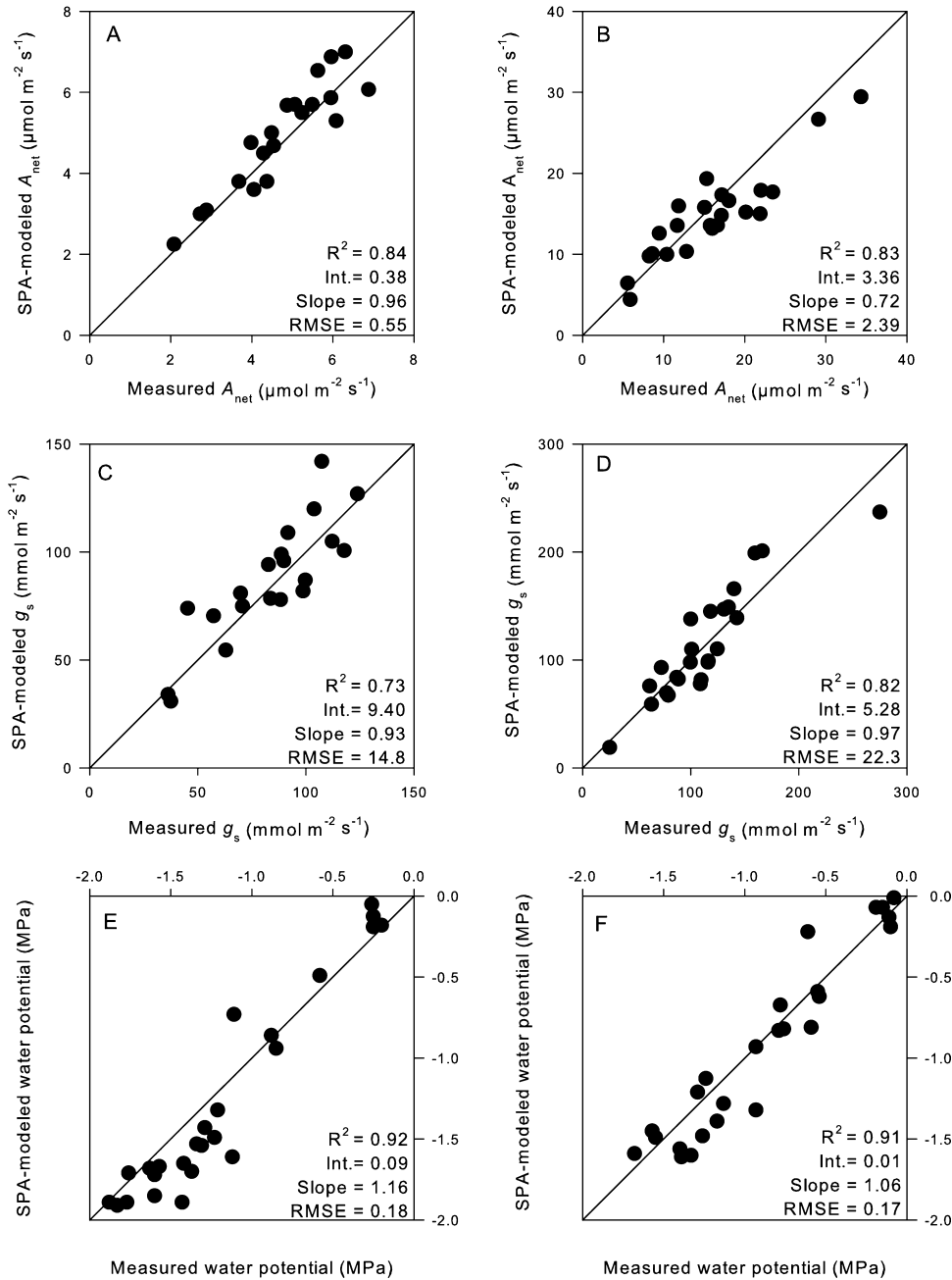
**Fig. 3.** (A) Maximum leaf-level switchgrass assimilation rates ( $A_{max}$ ) and (B) stomatal conductance ( $g_s$ ) measured over the 2010 growing season under ambient temperature and relative humidity conditions, with photosynthetic photon flux density of  $1600 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $\text{CO}_2$  concentration of  $380 \mu\text{mol mol}^{-1}$ . Rates are expressed as day of year (DOY) in the switchgrass-only treatments, and in the pine-switchgrass intercropped treatments where material from harvesting the previous rotation was retained (intercrop + biomass) or removed (intercrop - biomass). Values presented are means ( $N = 18$ ); error bars indicate the standard error of the mean.



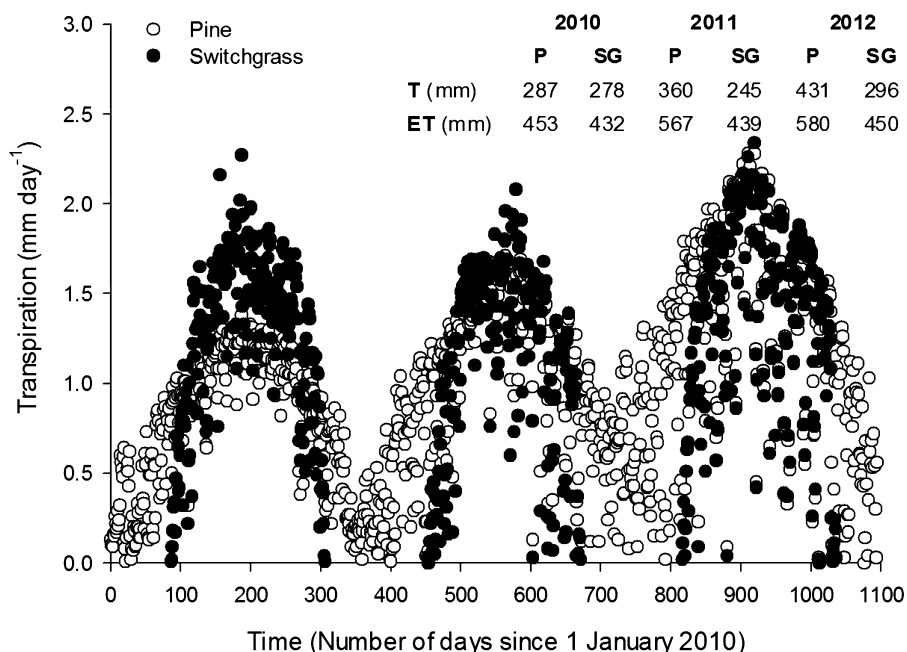
**Table 5**

Mean, *F*-statistic and *P*-value of comparisons between pure switchgrass and switchgrass intercropped with pine where material from harvesting the previous rotation was retained on site (intercrop + biomass) or removed (intercrop – biomass) for light saturated photosynthetic assimilation rate ( $A_{\text{sat}}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), the apparent quantum yield of photosynthesis ( $\alpha$ ,  $\mu\text{mol CO}_2 \mu\text{mol}^{-1}$  absorbed quanta), a parameter theta ( $\theta$ ) which describes the sharpness of the transition from light limitation to light saturation, and the rate of dark respiration ( $R_d$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), calculated from light-response curves, and the carboxylation efficiency ( $k$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ) and maximum Rubisco activity ( $V_{\text{cmax}}$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), estimated from *A*–*C*<sub>i</sub> response curves. Data were collected during the 2010 growing season.

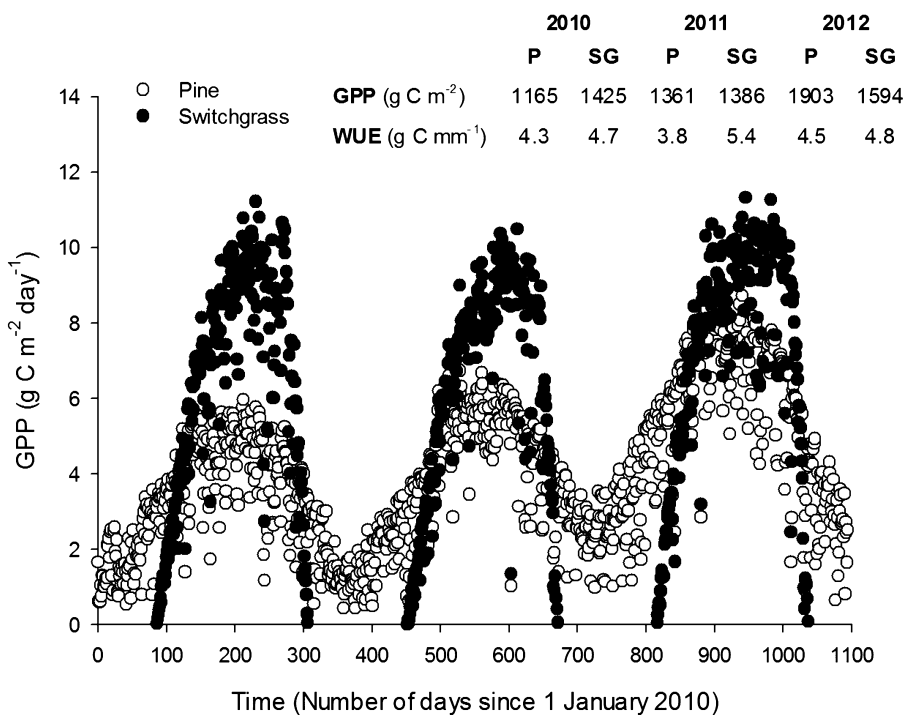
Parameter	Switchgrass	Intercrop + biomass	Intercrop – biomass	<i>F</i> -statistic	<i>P</i> -value
$A_{\text{sat}}$	25.6	32.6	28.3	0.91	0.534
$\alpha$	0.059	0.058	0.057	2.86	0.166
$\theta$	0.79	0.73	0.73	0.83	0.571
$R_d$	3.6	3.2	3.2	2.48	0.200
$k$	0.28	0.29	0.37	1.20	0.381
$V_{\text{cmax}}$	26.0	28.2	28.4	2.69	0.076



**Fig. 5.** Measured (*x*-axis) versus SPA model-simulated (*y*-axis) pine and switchgrass net assimilation ( $A_{\text{net}}$ ), stomatal conductance ( $g_s$ ), and leaf water potential ( $\psi$ ). Panels A, C and E are pine data and panels B, D and F are switchgrass data. Data points represent individual diurnal measurements and modeled values, averaged across treatment for each measurement date. 1:1 lines are indicated on the graphs. Summary statistics of model performance ( $R^2$ , the intercept (int.) and slope of the linear regressions between measured and simulated values, and the root mean square error (RMSE) are presented in each panel.



**Fig. 6.** SPA-modeled daily pine and switchgrass transpiration for 2010–2012. Modeled annual transpiration (T) and evapotranspiration (ET, which is the sum of transpiration, soil evaporation and canopy interception and evaporation rates) values are shown.



**Fig. 7.** SPA-modeled daily pine and switchgrass gross primary productivity (GPP) for 2010–2012. Modeled annual GPP values are shown, and water use efficiency (WUE), calculated as GPP/evapotranspiration. Pine WUE was calculated as the average annual GPP/evapotranspiration, whereas switchgrass WUE values were calculated over the growing season from March to November each year.

stomatal conductance, respectively). In addition, proximity to the nearest pine row did not affect switchgrass light-response parameters, carboxylation efficiency or maximum Rubisco activity in the intercropped treatments (Table 6).

### 3.6. Pine and switchgrass seasonal gas exchange and SPA model output

Seasonal pine and switchgrass gas exchange measured over midday (1000–1400 h) under ambient field conditions in 2011

and 2012 are presented in Fig. 4. The full range of diurnal gas exchange values is listed in Table 1. Both species followed a similar seasonal pattern for net assimilation and stomatal conductance (Fig. 4). On all measurement dates, switchgrass gas exchange rates were higher, but more variable than loblolly pine. Minimum midday leaf-level assimilation rates were measured in June 2011 ( $3.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for pine and October 2012 ( $10.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for switchgrass. Maximum assimilation rates were recorded in April 2012 ( $6.9$  and  $22.3 \mu\text{mol m}^{-2} \text{s}^{-1}$  for pine and switchgrass, respectively). Midday stomatal conductance values ranged from 62

**Table 6**

*F*-statistic and *P*-value results to determine whether distance from the nearest pine row affected switchgrass light saturated photosynthetic assimilation rate ( $A_{\text{sat}}$ ), the apparent quantum yield of photosynthesis ( $\alpha$ ), a parameter theta ( $\theta$ ) which describes the sharpness of the transition from light limitation to light saturation, and the rate of dark respiration ( $R_d$ ), calculated from light-response curves, and the carboxylation efficiency ( $k$ ) and maximum Rubisco activity ( $V_{\text{cmax}}$ ), estimated from  $A-C_i$  response curves. Data were collected during the 2010 growing season.

Parameter	<i>F</i> -statistic	<i>P</i> -value
$A_{\text{sat}}$	2.91	0.708
$\alpha$	1.79	0.882
$\theta$	0.70	0.519
$R_d$	1.13	0.619
$k$	0.21	0.802
$V_{\text{cmax}}$	0.17	0.808

to 124 mmol m<sup>-2</sup> s<sup>-1</sup> for pine and from 82 to 129 mmol m<sup>-2</sup> s<sup>-1</sup> for switchgrass.

As there were no significant treatment differences in gas exchange, parameter estimates from the light- and CO<sub>2</sub> response curves or other input variables used in the model (see Results section and Albaugh et al., 2012), parameter values were pooled across treatment and modeling estimates were based on pure pine, and pure switchgrass treatments. The SPA model fit these data well for leaf-level net assimilation,  $g_s$  and water potential, as shown by the high  $R^2$  values, low root mean square error and slopes close to 1 (Fig. 5A–F). Simulated switchgrass transpiration and GPP were low at the beginning and end of the growing season, but increased with temperature and growth of switchgrass (Figs. 6 and 7). Highest transpiration rates were recorded in June–August of each year, with maximum daily values of 1.4–2.3 mm for pine and 2.1–2.3 mm for switchgrass. The effect of Hurricane Irene is evident in our data (Day 240 in 2011), where wind gusts up to 80 km h<sup>-1</sup> were recorded and transpiration dropped to 0.03 mm (switchgrass) and 0.13 mm (pine) (Fig. 6). Modeled annual pine transpiration rates of 287, 360 and 431 mm were estimated for 2010, 2011 and 2012, respectively, with corresponding ET values of 453, 567 and 580 mm. Modeled annual switchgrass transpiration rates of 278, 245 and 296 mm were estimated for 2010, 2011 and 2012, respectively, with associated ET values of 432, 439 and 450 mm. Pine transpiration accounted for 63–74% of ET, whereas switchgrass transpiration was 56–66% of ET over the three-year simulation period. As we parameterized the SPA model for each species individually, we estimated total stand water use by summing the ground area covered by each species when intercropped, multiplied by the species-specific water use rates derived from the SPA model. Transpiration of competing vegetation growing in the area between the edge of the pine beds and the planted switchgrass swath was not quantified. Therefore, we used average understory transpiration (320 mm) reported by Domec et al. (2012) for a nearby young loblolly pine stand on the Lower Coastal Plain of North Carolina, as an estimate of competing vegetation water use in our stand. These calculations produced annual water use estimates of 636, 712 and 770 mm, respectively, for 2010, 2011 and 2012, in intercropped treatments.

Greatest simulated pine GPP was observed from June–August each year, with annual values of 1165, 1361 and 1903 g C m<sup>-2</sup> for 2010, 2011 and 2012, respectively (Fig. 7). Maximum switchgrass GPP occurred later, from July–September, with annual values of 1425, 1386 and 1594 g C m<sup>-2</sup>, respectively, for 2010, 2011 and 2012.

Whole-stand water use efficiency, calculated as GPP/evapotranspiration, was 4.3 (2010), 3.8 (2011) and 4.5 (2012) g C mm<sup>-1</sup> for pine (calculated from January to December each year), and 4.7 (2010), 5.4 (2011) and 4.8 g C mm<sup>-1</sup> (2012) for switchgrass (calculated over the switchgrass growing season from March to November each year).

#### 4. Discussion and conclusions

Our objectives were to measure the seasonal course of switchgrass gas exchange and to determine how intercropping with pines affects switchgrass physiology. We also assessed switchgrass photosynthesis response to changes in light intensity and CO<sub>2</sub> concentration, and parameterized the SPA model for pure pine and switchgrass at this site. There were no significant treatment effects on switchgrass gas exchange variables (photosynthesis and stomatal conductance). However, there were significant DOY effects for these variables and a significant treatment by DOY effect for photosynthesis.

It appears that the significant decrease in switchgrass photosynthetic rates in the intercropped–biomass treatment from July to August (DOY 195–230; Fig. 3, Table 4) was driven mainly by low VSWC (Fig. 2), caused by higher soil evaporation rates. Harvest residue retention has been shown to result in higher VSWC during the growing season and to act as a buffer against large fluctuations in soil temperature (Entry et al., 1987; Smethurst and Nambiar, 1990; Roberts et al., 2005). In our study, soil VWC and temperature at each depth (10 and 30 cm) were calculated from one soil moisture probe per plot, and we were not able to detect a significant treatment or treatment × DOY effect, possibly due to the limited number of probes. Decreases in switchgrass photosynthesis due to limited water availability have been demonstrated by Wullschlegel et al. (1996), who reported a 70% reduction in field photosynthetic rates in the middle of the growing season in southeastern U.S., which they attributed to a lack of precipitation. Furthermore, low soil VWC significantly decreased switchgrass photosynthetic rates in a lysimeter study in the southern U.S. (Sanderson and Reed, 2000).

Under conditions of non-limiting soil water in October (DOY 279), where VSWC measured 0.454 m<sup>3</sup> m<sup>-3</sup> (Fig. 2), we suggest that switchgrass gas exchange was driven by other environmental or plant variables, such as air and soil temperature, and leaf phenology and ontogeny. According to Ku et al. (1978), the decline in switchgrass photosynthetic rates as the growing season progresses can be attributed to changes in stomatal conductance associated with lower night temperatures. Ku et al. (1978) observed that cool nights slowed the opening of stomata the following day, leading them to suggest that photosynthesis is strongly regulated by stomatal resistance in this species. In addition, Sage et al. (2011) reported that at cooler temperatures, Rubisco capacity is limited in C<sub>4</sub> plants. Rubisco has a Q<sub>10</sub> near 2.2, therefore its turnover capacity decreases rapidly with temperature (i.e. more than half for every 10 °C decrease in temperature). At cooler temperatures, Rubisco capacity declines below the capacity of the C<sub>4</sub> cycle and ribulose-1,5-bisphosphate regeneration, due to the low amount of Rubisco in C<sub>4</sub> versus C<sub>3</sub> leaves (Sage et al., 2011). In our study, coldest average nighttime temperatures (15.3 °C) were recorded in October 2010 (compared to 23.7, 23.5 and 23.5 °C measured in June, July and August, respectively), and more than 55% of the most recent fully-formed leaves of measured switchgrass tillers across all treatments were undergoing senescence, or had senesced at this time (Albaugh et al., 2012). It is likely that leaf senescence combined with cool temperatures led to the low switchgrass photosynthetic rates measured at the end of the season.

To our knowledge, the current study is the first to report detailed leaf gas exchange data for switchgrass grown as a forest biofuel intercrop on an acidic soil. Previous physiological studies that have reported leaf-level gas exchange data for switchgrass have been mainly from upland varieties (predominantly Cave-in-Rock, compared to the lowland ecotype Alamo used in this study), or from the U.S. Midwest, mainly from pure switchgrass stands, some of which were established for screening purposes only. There are some data from field trials (e.g. Skeel and Gibson, 1996; Wullschlegel et al., 1996; Dohleman et al., 2009), but gas exchange data have not been

documented on this ecotype grown on the Lower Coastal Plain of North Carolina, nor when intercropped as a biofuel with pine. Importantly, our study was on an intensively managed forest plantation site with low soil pH, less uniform seed bed conditions, and history of minimal management compared to an agricultural setting. Switchgrass gas exchange data were consistent with values reported in the literature, where maximum photosynthetic rates of up to  $34.9 \mu\text{mol m}^{-2} \text{s}^{-1}$  have been reported (e.g. [Skeel and Gibson, 1996](#); [Wullschlegel et al., 1996](#); [Anderson and Reed, 2000](#); [Dohleman et al., 2009](#)), compared to  $32.7 \mu\text{mol m}^{-2} \text{s}^{-1}$  in our study. Stomatal conductance ranged from 104 to  $252 \text{mmol m}^{-2} \text{s}^{-1}$  which is within the range of 33 to  $356 \text{mmol m}^{-2} \text{s}^{-1}$  reported in the literature ([Skeel and Gibson, 1996](#); [Dohleman et al., 2009](#)).

Parameter estimates from the switchgrass light response curves were not affected by treatment ([Table 5](#)). The initial slope of the light response curve of  $\text{CO}_2$  fixation may be described as the apparent quantum yield or efficiency of light use by photosynthesis ([Collatz et al., 1992](#); [von Caemmerer, 2000](#)). Quantum yields of  $\text{C}_4$  plants are constant at different light intensities, and are independent of temperature regimes and  $\text{CO}_2$  concentrations ([Ehleringer and Björkman, 1977](#); [Björkman, 1981](#)). The quantum yield estimated in our study was  $0.059 \mu\text{mol CO}_2 \mu\text{mol}^{-1}$  absorbed quanta compared to literature values for  $\text{C}_4$  species which range from  $0.045$ – $0.061 \mu\text{mol CO}_2 \mu\text{mol}^{-1}$  absorbed quanta ([Ehleringer and Björkman, 1977](#); [Ehleringer and Pearcy, 1983](#); [Monson et al., 1982](#); [Ehleringer et al., 1997](#)). The initial slope of the  $\text{CO}_2$  response curve is proportional to phosphoenolpyruvate (PEP) carboxylase activity (referred to as carboxylation efficiency,  $k$ ), whereas the saturated rate is proportional to Rubisco activity ( $V_{\text{cmax}}$ ) ([von Caemmerer, 2000](#)). Analysis of switchgrass  $A-C_i$  curves revealed there were no treatment effects ([Table 5](#)). Pooling data across all treatments yielded  $k$  and  $V_{\text{cmax}}$  estimates of  $0.31 \pm 0.02 \text{mol m}^{-2} \text{s}^{-1}$ , and  $27.6 \pm 1.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. PEP carboxylase and Rubisco activity vary with leaf age, nitrogen nutrition, temperature, leaf water potential and light environment during growth ([Usada, 1984](#); [Hunt et al., 1985](#); [Wong et al., 1985](#); [Sage et al., 1987](#); [Polley et al., 1992](#); [Massad et al., 2007](#)). Reported values of  $k$  and  $V_{\text{cmax}}$  for  $\text{C}_4$  species range from  $0.11$  to  $1.02 \text{mol m}^{-2} \text{s}^{-1}$ , and  $16.0$  to  $42.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively, over a wide range of growing conditions, leaf temperatures and nitrogen concentrations ([Collatz et al., 1992](#); [Polley et al., 1992](#); [Akkasaeng, 1993](#); [Massad et al., 2007](#); [Ripley et al., 2010](#)).

No significant treatment effects were observed in switchgrass gas exchange, VSWC or temperature, or parameter estimates from the light- and  $\text{CO}_2$  response curves during 2010. Similarly, switchgrass biomass production did not differ between treatments ([Albaugh et al., 2012](#)). However, this study was a large field experiment, with a high variance in gas exchange measurements ([Figs. 3 and 4](#)). A field experiment of this size is limited by sampling replication for practical reasons.

Analyses based on the stratified random sampling approach used in the intercropped treatments revealed that at this stage of stand development, proximity to a pine row did not affect any switchgrass gas exchange variable or parameter estimates derived from the light- and  $\text{CO}_2$  response curves ([Table 6](#)). However, we anticipate that as this intercropped system develops over time, availability of resources such as light, water or nitrogen may change, with the potential to impact switchgrass physiology, especially  $k$  and  $V_{\text{cmax}}$  values.

Evaluation of SPA model outputs against field measurements of leaf-level physiology indicated generally good agreement ([Fig. 5](#)). Modeled annual stand-level water use for pine ranged from 287 to 431 mm (transpiration) and 453 to 580 mm (evapotranspiration), and for switchgrass, from 245 to 296 mm (transpiration), and 432 to 450 mm (evapotranspiration) over the three-year period ([Fig. 6](#)). Model-simulated pine water use is similar to transpiration rates

of 223 and 357 mm reported by [Samuelson and Stokes \(2006\)](#) and [Samuelson et al. \(2008\)](#) for four- and five-year old loblolly pine stands. Annual simulated pine GPP ranged from 1165 to  $1903 \text{g C m}^{-2}$  ([Fig. 7](#)), which is comparable to the  $1220$ – $2550 \text{g C m}^{-2}$  range reported for loblolly pine aged 8–14 years growing in southeastern U.S. ([Lai et al., 2002](#); [Gough et al., 2004](#); [Maier et al., 2004](#)). Annual simulated switchgrass GPP ranged from 1386 to  $1594 \text{g C m}^{-2}$ . No information could be found in the literature on stand-level water use or GPP for switchgrass in the southeast U.S. However, SPA model outputs from our study are comparable to literature values from the midwest and northeast U.S., where evapotranspiration rates of 300 mm ([McIsaac et al., 2010](#)), 474 mm ([Skinner and Adler, 2010](#)), 498 mm ([Le et al., 2011](#)) and 764 mm ([Hickman et al., 2010](#)) were reported for Cave-in-Rock switchgrass variety (as opposed to Alamo used here). In addition, [Wagle and Kakani \(2012\)](#) measured 450 mm evapotranspiration in Alamo switchgrass in the southwest-U.S. from May to mid-November. Annual GPP rates quantified by [Skinner and Adler \(2010\)](#) ranged from 914 to  $940 \text{g C m}^{-2}$  for the first four years after establishment of Cave-in-Rock switchgrass variety in northeast U.S. The higher GPP rates in our study were likely due to the high-producing lowland Alamo ecotype compared to the upland Cave-in-Rock ([McLaughlin et al., 1999](#)), and the warmer temperatures and longer growing season at our site. [Wagle and Kakani \(2012\)](#) reported GPP of  $1139 \text{g C m}^{-2}$  over the period May to mid-November for Alamo switchgrass in southwest U.S. Whole-stand water use efficiency (WUE), calculated as GPP/evapotranspiration over the period March to November each year, ranged from  $4.7$  to  $5.4 \text{g C mm}^{-1}$ . These values are comparable to maximum WUE reported by [Skinner and Adler, 2010](#), which ranged from  $3.3$  to  $4.1 \text{g C mm}^{-1}$  for Cave-in-Rock switchgrass in northeast U.S., and  $2.9$  to  $3.3 \text{g C mm}^{-1}$  for Alamo variety growing under drought conditions in southwest U.S. ([Wagle and Kakani, 2012](#)). The close match between literature values and SPA model predictions of water use and GPP is encouraging as the model provides realistic estimates of water use and C exchange for young loblolly pine and a  $\text{C}_4$  species. This suggests the model may have utility to aid in climate change scenario analyses by manipulating inputs of environmental conditions such as  $\text{CO}_2$  concentration, temperature and rainfall amount and distribution.

We parameterized an ecosystem model for pine and switchgrass and used this model to provide estimates of water use over a three-year period. Our calculations produced annual water use estimates of 636, 712 and 770 mm for intercropped treatments in 2010, 2011 and 2012, respectively. A comparison of these values against rainfall totals of 1252, 1158 and 1232 mm for each year yielded respective surplus water amounts of 616, 446 and 462 mm, for 2010, 2011 and 2012. This indicates that at the time, there was sufficient water at this site to support growth of both species. Over the three-year period, intercropped treatments used 21% more water than pine grown alone, and 60% more water than where switchgrass was grown alone.

This study is the first to characterize switchgrass physiology when grown as a biofuel intercrop in experimental forested settings on the Lower Coastal Plain of North Carolina, and the first to provide stand-level estimates of water use and C exchange of switchgrass in the southeast U.S.

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