

Hydraulic limitation not declining nitrogen availability causes the age-related photosynthetic decline in loblolly pine (*Pinus taeda* L.)

J. E. DRAKE¹, L. M. RAETZ², S. C. DAVIS^{2,3} & E. H. DELUCIA^{1,2,3,4}

¹Program of Ecology, Evolution, and Conservation Biology, University of Illinois, Urbana-Champaign, IL 61802, USA,

²Department of Plant Biology, University of Illinois, Urbana-Champaign, IL 61802, USA, ³Energy Biosciences Institute, University of Illinois, Urbana-Champaign, IL 61802, USA and ⁴Institute of Genomic Biology, University of Illinois, Urbana-Champaign, IL 61802, USA

ABSTRACT

Declining net primary production (NPP) with forest age is often attributed to a corresponding decline in gross primary production (GPP). We tested two hypotheses explaining the decline of GPP in ageing stands (14–115 years old) of *Pinus taeda* L.: (1) increasing N limitation limits photosynthetic capacity and thus decreases GPP with increasing age; and (2) hydraulic limitations increasingly induce stomatal closure, reducing GPP with increasing age. We tested these hypotheses using measurements of foliar nitrogen, photosynthesis, sap-flow and dendroclimatological techniques. Hypothesis (1) was not supported; foliar N retranslocation did not increase and declines were not observed in foliar N, leaf area per tree or photosynthetic capacity. Hypothesis (2) was supported; declines were observed in light-saturated photosynthesis, leaf- and canopy-level stomatal conductance, concentration of CO₂ inside leaf air-spaces (corroborated by an increase in wood δ¹³C) and specific leaf area (SLA), while stomatal limitation and the ratio of sapwood area (SA) to leaf area increased. The sensitivity of radial growth to inter-annual variation in temperature and drought decreased with age, suggesting that tree water use becomes increasingly conservative with age. We conclude that hydraulic limitation increasingly limits the photosynthetic rates of ageing loblolly pine trees, possibly explaining the observed reduction of NPP.

Key-words: A:c; Granier; hydraulic limitation hypothesis; sap-flow.

INTRODUCTION

Net primary production (NPP) is the ultimate source of energy for all food webs and the starting point for ecosystem services that make human life possible. Thus, considerable scientific attention has been direct to quantifying NPP at a number of scales (Field *et al.* 1998; Ollinger, Aber & Federer 1998; Huston & Wolverton 2009), and determining

Correspondence: E. H. DeLucia. Fax: +1 217 244 7246; e-mail: delucia@illinois.edu

the biotic and abiotic controls over this important flux (Hamilton *et al.* 2002; Richardson *et al.* 2007; McCarthy *et al.* 2009). Forests comprise one quarter of global NPP (Field *et al.* 1998), which corresponds to a flux of C more than four times the rate of anthropogenic emissions (Denman *et al.* 2007). Thus, forest production is a large and important component of global C cycling.

A decline in forest NPP with increasing age is commonly observed (reviews by Ryan, Binkley & Fownes 1997a; Pregitzer & Euskirchen 2004; DeLucia *et al.* 2007); however, the mechanism causing this decline is still a matter of debate. The initial hypothesis that autotrophic respiration (R_a) increases as non-photosynthetic biomass increases, causing a decreasing fraction of gross primary production (GPP) to be available for NPP with age (Moller, Muller & Nielsen 1954; Yoda *et al.* 1965; Kira & Shidei 1967; Whittaker & Woodwell 1967; Odum 1969) has not been supported (Ryan & Waring 1992; Harrington & Fownes 1995; Ryan *et al.* 1996, 2004; Ryan, Lavigne & Gower 1997b). This led to research into how the decline in NPP could be driven by a decline in GPP because of increasing hydraulic (Yoder *et al.* 1994; Ryan & Yoder 1997; Ryan, Phillips & Bond 2006) or nutrient (Gower, McMurtrie & Murty 1996; Murty, McMurtrie & Ryan 1996) limitations to photosynthesis. Hydraulic limitation of photosynthesis is thought to increase with tree age as it becomes increasingly difficult to transport water to the leaves of old, tall trees, causing stomata to close to avoid cavitation (Ryan *et al.* 2006). Nitrogen limitation may also increase with forest age if the immobilization of soil N in woody biomass causes a reduction of available nutrient pools sufficient to cause a reduction in foliar area or photosynthetic capacity, causing photosynthesis to decline (Luo *et al.* 2004; Johnson 2006).

Previously, we demonstrated that NPP declines strongly with age in loblolly pine (*Pinus taeda* L.) forests, driven exclusively by an 80% decline in pine wood production (Drake *et al.* 2010). Sap-flow-derived estimates of GPP and measurements of stem respiration indicated that the decline in pine NPP was driven by a decline in pine GPP, not an increase in R_a (Drake *et al.* 2010).

Here, we tested two hypotheses regarding an age-related decline in GPP: (1) increasing N limitation increasingly limits photosynthetic capacity and thus decreases GPP with increasing age; and (2) increasing hydraulic limitation increasingly induces stomatal closure and thus decreases GPP with increasing age. If hypothesis (1) was true, we expected to observe declining foliar N%, declining leaf area per tree, increased translocation of N before leaf abscission and declining photosynthetic capacity as defined by the maximum rate of carboxylation ($V_{c,\max}$) and the maximum rate of electron transport (J_{\max} ; Long & Bernacchi 2003). If hypothesis (2) was true, then we expected to observe declines in stomatal conductance, reduced specific leaf area (SLA) driven by reduced turgor-induced cellular expansion, decreased concentrations of CO₂ inside leaves (c_i) relative to the CO₂ concentrations in the atmosphere around leaves (c_a), reduced canopy conductance and compensation of hydraulic limitation through an increasing ratio of sapwood area (SA) to leaf area (Ryan *et al.* 2006). Many of these predictions were tested with leaf-level gas exchange and stem sap-flow measurements. We also used a dendroclimatological approach to investigate correlations between radial growth and climate, with the prediction that increased hydraulic limitation would make radial growth more strongly correlated with inter-annual variation in temperature and drought with increasing age.

METHODS

Study sites

We identified a chronosequence of 12 forest stands that were similar in all respects except the age of the dominant loblolly pine (*P. taeda*) trees. All sites were within the Kinston and Durham divisions of the Duke Forest, located in Durham County, NC, USA (centred near 36°00'N 78°58'W). Sites were chosen to have similar land use, initial stocking densities, management history and soil types

(J. Edeburn, personal communication), but the age of the pines varied from 14 to 115 years, including the oldest loblolly pines present in this region (Table 1). The Helena soil series was chosen for site selection because it closely matches the soil at the nearby Duke Free-air CO₂ Enrichment (FACE) site (Hamilton *et al.* 2002), enabling comparisons between data presented here and this 26-year-old forest that lies ~5 km to the west. The maximum distance between stands was 6 km. Detailed descriptions of the site selection criteria, stand characteristics and production measurements are provided in Drake *et al.* (2010).

Pine SA was measured in all stands using an allometric relationship between diameter at breast height (DBH) and SA; the allometry was derived by coring 41 trees of varying size, with two perpendicular cores per tree, and differentiating sapwood with a 10% ferric chloride solution [SA (cm²) = 18.24*DBH (cm) - 258.05; $r^2 = 0.81$, $P < 0.001$, $n = 41$].

Ecosystem leaf area index (*LAI*, m² leaf area m⁻² ground area) was measured at 10 locations within each stand with a canopy analyser (LAI 2000, Li-Cor, Lincoln, NE, USA) at approximately monthly intervals. Measurements of *LAI* were corrected for foliar clumping and woody interception (Chen 1996) as in Drake *et al.* (2010). The relative contribution of pines and hardwoods to total *LAI* was estimated from litterfall collections (Drake *et al.* 2010). Pine *LAI* was estimated as the difference between summer maximum ecosystem *LAI* (optical method) and hardwood *LAI* (litterfall method). The ratio of pine SA to pine leaf area was calculated at the stand level by summing the SA of all pines and dividing by pine *LAI* (Table 1).

Gas exchange

Foliage gas exchange was measured with cross-calibrated, open gas exchange systems (model 6400, Li-Cor Biosciences) under saturating irradiance (1500 μmol PAR m⁻² s⁻¹, 6400-02B red/blue light source, 2 × 3 cm chamber,

Table 1. Stand characteristics of 12 *Pinus taeda* forests of varying age in North Carolina

Age (years)	DBH (cm)	Height (m)	Stand maximum <i>LAI</i> (m ² m ⁻²)			
				Pine <i>LAI</i> (m ² m ⁻²)	Pine SA (cm ² m ⁻²)	Pine SA/ <i>LAI</i> (cm ² m ⁻²)
14	18 (0.5)	15 (0.4)	4.6 (0.2)	4.2 (0.2)	13.0	3.1
19	17 (0.4)	14 (0.4)	4.7 (0.2)	3.8 (0.2)	16.4	4.3
22	20 (0.6)	19 (0.7)	4.7 (0.2)	3.8 (0.2)	21.1	5.6
36	24 (0.8)	27 (1.3)	5.0 (0.2)	3.2 (0.2)	14.1	4.4
42	28 (1.3)	25 (0.8)	4.8 (0.2)	2.0 (0.2)	11.4	5.7
53	33 (1.1)	30 (1.2)	4.7 (0.2)	2.5 (0.2)	11.5	4.6
71	46 (2.1)	33 (0.7)	4.0 (0.1)	2.3 (0.1)	13.1	5.6
74	48 (2.7)	36 (1.3)	4.1 (0.2)	2.6 (0.2)	11.6	4.4
79	41 (1.3)	34 (1.7)	4.4 (0.2)	2.8 (0.2)	15.7	5.5
97	59 (2.0)	40 (3.3)	5.2 (0.2)	2.8 (0.2)	13.9	4.9
114	54 (2.2)	43 (0.8)	5.0 (0.2)	2.5 (0.2)	14.6	5.7
115	54 (1.9)	39 (1.5)	4.2 (0.2)	1.8 (0.2)	12.4	6.8

Values are the mean (± 1 SE) of 30–150 measurements for DBH and 10 measurements for height and *LAI*. *LAI* refers to projected leaf area index, and SA refers to sapwood area per unit ground area. SA and SA/*LAI* were calculated at the stand level, and thus have no subreplicate measurements.

Li-Cor Biosciences). The midpoint of three fascicles (i.e. nine needles) was enclosed in the chamber; leaks formed by clamping the flat chamber gaskets onto irregular needle surfaces were sealed with Permagum (Virginia KMP, Broadview, IL, USA), and each sample was verified to be leak tight by exhaling on the chamber. Relationships between net photosynthesis (A_{net}) and the CO₂ partial pressure (c_i) inside needle air-spaces (i.e. $A:c_i$ curves) were measured by recording A_{net} at the following ambient concentrations of CO₂ (c_i) in the reference cell of the gas exchange system: 400, 250, 150, 50, 400, 550, 750, 1000, 1200, 1400 and 1800 ppm. All $A:c_i$ curves were analysed using the PS-fit program (version 7.3, Bernacchi *et al.* 2002; Bernacchi, Pimentel & Long 2003; Long & Bernacchi 2003). Parameters derived from this fitting procedure (e.g. $V_{c,\text{max}}$ and J_{max}) were reported at 25 °C.

Loblolly pine trees carry needles for 18 months (Zhang & Allen 1996), and thus have cohorts of needles that were produced in the current year and the previous year. The previous year's foliage was measured in all cases, as these needles were more likely to have experienced conditions of water stress and would be more likely to display evidence of hydraulic limitation. Additionally, old needle cohorts often maintain similar photosynthetic activity relative to new foliage (Greenway, Macdonald & Lieffers 1992; Crous & Ellsworth 2004), and thus contribute substantially to whole stand photosynthesis.

As these sites lacked canopy access, we sampled canopy foliage with a shotgun (Liu, Ellsworth & Tyree 1997; Thomas *et al.* 2008). To investigate potential artefacts of measuring gas exchange on detached branches, especially the possibility of reduced stomatal conductance (g_s), we conducted two experiments using the canopy access towers at the Duke FACE site where gas exchange of attached and detached branches was compared; trees at this site averaged 22 cm DBH and 20 m tall in 2008. In the first experiment, needles were placed in gas exchange chambers while maintaining the connection to the branch and until light-saturated photosynthesis (A_{sat}) reached steady state, after which the branch was detached from the tree and A_{sat} was measured every minute over the next hour. We performed these measurements throughout the course of 2 d to identify the optimal time of sampling. In the second experiment, $A:c_i$ curves were measured on attached needles in the mid-morning; the branch was then detached and a second $A:c_i$ curve was measured after 10 min. Five pairs of $A:c_i$ curves were measured in this manner to investigate the potential bias of branch sampling on gas exchange measurements.

Having confirmed the efficacy of shotgun sampling (see Results), 8–10 upper canopy branches were shot down from 9 of the 12 sites in the midmorning through the early afternoon (1000 to 1300 EST). Three sites were excluded because their proximity to roads or public dwellings made shotgun sampling prohibitively dangerous. Gas exchange was measured immediately following shotgun sampling. One site was measured per day from July 12th to July 23rd, 2008. We controlled the block temperature of the gas exchange systems at 30 °C at all sites; average leaf

temperature was between 29.9 to 32 °C and did not vary significantly with age (regression, $P > 0.1$). We did not control water vapour pressure in the cuvette which varied from 1.29 to 1.99 kPa, but did not vary systematically with stand age (regression, $P > 0.1$). These results indicate that day-to-day variation in climate conditions was small. The needle area inside the chamber was photographed, and projected leaf area was measured using the ImageJ program (Abramoff, Magelhaes & Ram 2004). Afterward, needles were dried for 2 d at 60 °C, ground and analysed for carbon and nitrogen concentrations using micro-Dumas combustion elemental analysis (ECS 4010, Costech Analytical, Valencia, CA, USA). Gas exchange rates were reported on a projected area basis, and SLA was measured as projected needle area per dry mass (cm² g⁻¹).

Nitrogen translocation and leaf area

Fresh litterfall was collected from 10 0.22 m² litter traps per plot 3 d after the traps were emptied to ensure that litter had not experienced precipitation. Percent N retranslocation was calculated as $(N_{\text{fresh}} - N_{\text{litter}})/N_{\text{fresh}}$ as in Finzi *et al.* (2001), where N_{fresh} was the N concentration in fresh needles obtained by shotgun, and N_{litter} was the N concentration in freshly abscised needle litterfall.

In addition to affecting foliar N concentrations, increasing N limitation has the potential to reduce the leaf area of individual trees (Ryan *et al.* 2004). Pine LAI at the ecosystem level declined from 4.1 to 2.2 m² m⁻² from 15 to 115 years old, but total ecosystem LAI remained constant at ~4.5 for all ages because of the accumulation of later successional hardwood foliage (Table 1, Drake *et al.* 2010). To distinguish whether increasing N limitation reduced the leaf area supported by each individual tree, or if pine LAI declined because self-thinning reduced pine stem density, we quantified pine leaf area per individual by dividing summer pine LAI by stem density and compared these values to those predicted by an equation relating DBH to leaf area derived from destructive sampling at the Duke FACE site (Naidu, DeLucia & Thomas 1998).

Sap-flow

We instrumented eight pine trees in five of the 12 stands (14, 19, 36, 70 and 97 years of age) with thermal dissipation sap-flow probes (Granier 1987). We estimated radial and circumferential variation in J_s (Phillips, Oren & Zimmerman 1996; Ewers & Oren 2000; Wullschleger & King 2000) with additional measurements on the north and south sides (0–20 mm depth) and at 20–40 mm depth (north side) on two pines per stand. There was no difference between J_s measured in the outer 20 mm on the north-and south-facing sides ($P > 0.1$), so we did not apply circumferential corrections. We accounted for a measured decline in J_s with increasing sapwood depth as in Phillips *et al.* (1996) and Schäfer *et al.* (2002). Atmospheric humidity and temperature were measured with a capacitive relative humidity sensor and a thermistor (Vaisala HMP 35C; Campbell

Scientific, Logan, UT, USA) suspended in the upper third of the canopy in each stand, and vapour pressure deficit (VPD) (D) was calculated from relative humidity and air temperature. Leaf transpiration (E_l) and canopy conductance (G_c) were calculated using monthly measurements of LAI and a site-specific relationship between DBH and SA (Drake *et al.* 2010) as in Ewers *et al.* (2001).

Wood $\delta^{13}\text{C}$ composition

We measured the stable carbon isotope composition ($\delta^{13}\text{C}$) of wood to investigate if c_i/c_c measured by gas exchange was characteristic of the long-term behaviour of these trees. Biomass $\delta^{13}\text{C}$ values are often used as long-term integrators of c_i/c_c (Farquhar, Oleary & Berry 1982; Katul, Ellsworth & Lai 2000; Dawson *et al.* 2002) because the relative importance of isotopic fractionation by ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) carboxylation (fractionation of $\sim 27\text{\textperthousand}$) versus diffusion through the stomatal pore (fractionation of $4.4\text{\textperthousand}$) varies with c_i/c_c . Previously, we reported wood $\delta^{13}\text{C}$ values of the last 5 years of growth of eight pines per site (Drake *et al.* 2010); we include these data as an independent reference for the measurements of c_i/c_c derived from gas exchange. Wood was sampled with tree cores, dried, ground and analysed for $\delta^{13}\text{C}$ at the University of Illinois using an elemental analyser (ECS 4010; Costech Analytical) coupled to a Conflo IV interface (Thermo, Bremen, Germany) and a Delta-V advantage isotope ratio mass spectrometer (Thermo). The precision calculated from 12 samples run in duplicate was $0.09\text{\textperthousand}$, and the average measured deviation of an in-house isotopic reference material was $<0.05\text{\textperthousand}$ of its known value ($n = 5$).

Tree ring analysis

Twenty canopy trees were cored per site, with two cores at perpendicular angles per tree. The cores were sanded, imaged using a flatbed scanner (Epson Perfection V700 Photo; Epson, Rockford, IL, USA) and ring widths for the past 30 years were quantified using an image analysis program (Windendro; Regent Instruments Inc, Nepean, Ontario, Canada) and averaged by tree. Ring widths were detrended and built into site-level chronologies using standard dendrochronology techniques and the dplR package (R Development Core Team 2007; Bunn 2008). Detrended ring widths were then regressed against climate variables using DENDROCLIM (Franco & Kishor 2004), which determines the strength of the relationship between climate variables and annual ring width, and evaluates the significance of these correlations with bootstrap resampling techniques. Monthly average temperature and Palmer drought severity index (PDSI) for the past 30 years in Durham County were obtained from the National Climatic Data Center (<http://www1.ncdc.noaa.gov/pub/data/cirs/>).

Data analysis

Statistical analyses were performed using the MIXED and REG procedures of the SAS system (SAS 9.1; SAS

Institute, Cary, NC, USA). The assumptions of homoskedasticity and normality of residuals were checked using the UNIVARIATE and REG procedures. Non-linear regressions were fit in SigmaPlot (version 10.0; Systat Software, San Jose, CA, USA). Stands were treated as the unit of replication in all cases ($n = 9$ for gas exchange, fluorescence and SLA; $n = 12$ for wood isotopes and dendroclimatology; $n = 5$ for sap-flow).

RESULTS

Validation of gas exchange measurements

Detached branches sampled in the late afternoon exhibited a strong decline in g_s once harvested, while branches sampled in the mid-morning showed no change in g_s for >40 min (Fig. 1a), which was sufficient to measure an $A:c_i$

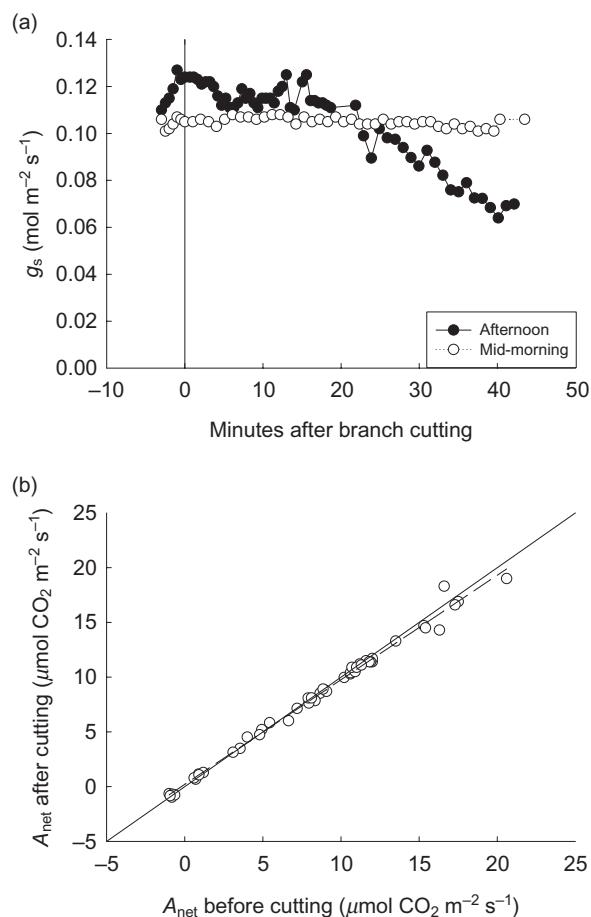


Figure 1. Validation of gas exchange measurements on detached branches of loblolly pine (*Pinus taeda*) measured using the canopy access towers in the ambient CO_2 plots at the Duke Free-air CO_2 Enrichment (FACE) site. Representative time-courses of stomatal conductance to H_2O (g_s) under light saturation following branch detachment (a). Branches were detached at time zero. Compilation of five pairs of $A:c_i$ curves performed before and after detaching branches (b). The solid line is the 1:1 relationship. The dashed line is the best linear fit: $y = 0.21 + 0.96x$, $P < 0.001$, $r^2 = 0.99$.

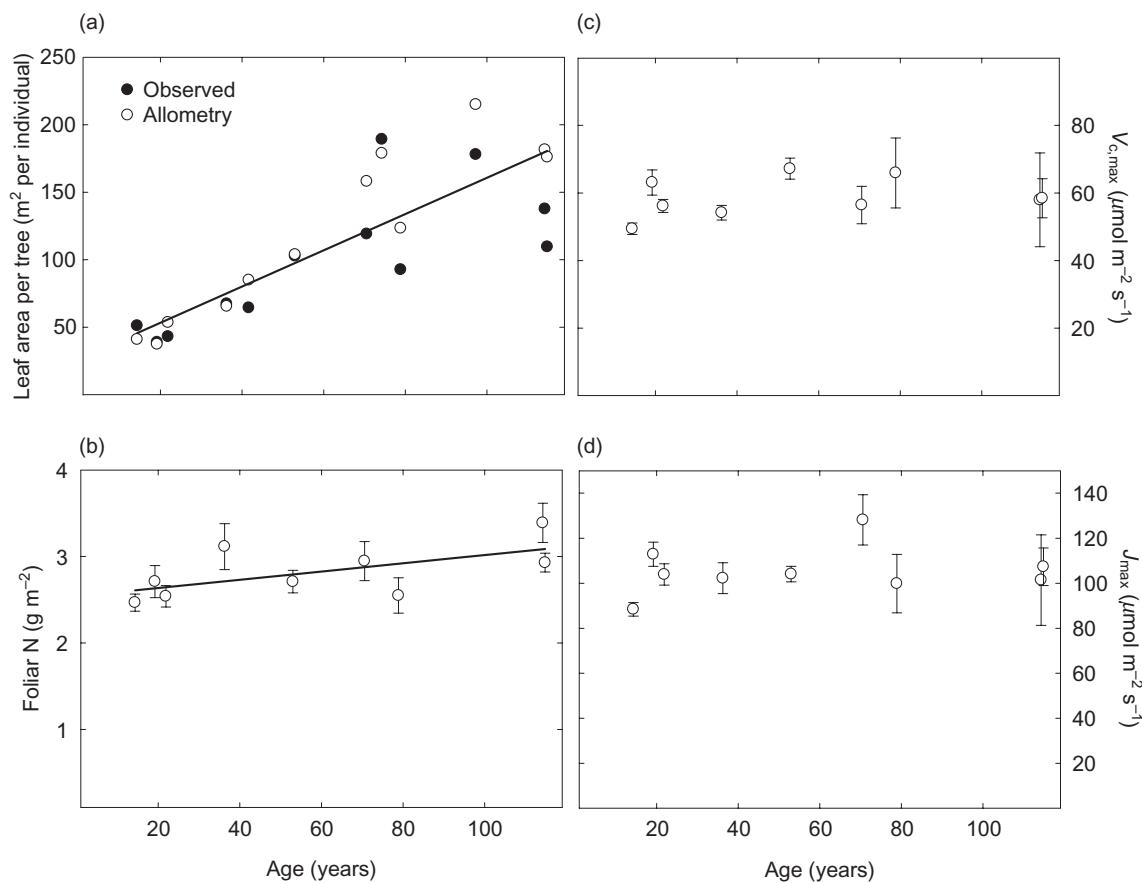


Figure 2. Leaf area and photosynthetic capacity of loblolly pine (*Pinus taeda*) needles in relation to stand age. Leaf area per tree (a) as observed and predicted from an equation relating leaf area to diameter at breast height (DBH). There was no difference in the slope or intercept between categories, so a single regression was used; $y = 26.48 + 1.34x$, $P < 0.001$, $r^2 = 0.69$. Foliar nitrogen concentrations (b) increased marginally with increasing age; $y = 2.54 + 0.005x$, $P = 0.08$, $r^2 = 0.28$. Carboxylation capacity ($V_{c,\text{max}}$; c) and the maximum rate of electron transport (J_{max} ; d) did not vary significantly with age. All error bars are ± 1 SE of 8–10 measurements per site.

curve. $A:c_i$ curves measured on attached and detached foliage showed a strong correlation between corresponding rates of photosynthesis (Fig. 1b, detached $A_{\text{net}} = 0.2 + 0.95^*$ attached A_{net} , $P < 0.001$, $r^2 = 0.99$, slope not significantly different than 1, $P > 0.5$). Additionally, $V_{c,\text{max}}$ and J_{max} derived from these measurements did not differ between attached or detached foliage (paired t -test, $P > 0.3$ for $V_{c,\text{max}}$, $P > 0.1$ for J_{max}). Thus, we concluded that $A:c_i$ curves could be measured on detached foliage without measurement artefacts as long as the branches were detached before 1400 h EST.

N limitation hypothesis

While ecosystem-level pine LAI declined with age (Table 1; Drake *et al.* 2010), leaf area per individual tree increased with age along the trajectory predicted by an equation derived from destructive measurements at the Duke FACE site (Fig. 2a; Naidu *et al.* 1998). There was a marginally significant increase in foliar N (Fig. 2b, $P = 0.08$) and no evidence of a systematic change in

photosynthetic capacity with increasing age (Fig. 2c,d) with no detectable change in $V_{c,\text{max}}$ or J_{max} . There was no detectable change in the retranslocation of N (Table 2).

Hydraulic limitation hypothesis

Light-saturated photosynthesis declined by ~25% with increasing age, from 12 to 9 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at 15 and 115 years of age, respectively (Fig. 3a). There was a corresponding age-related decline of ~40% in stomatal conductance, from 0.15 to 0.09 mol $\text{H}_2\text{O m}^{-2} \text{ s}^{-1}$ (Fig. 3b). The decline in conductance led to a decline in c_i (Fig. 3c) and an increase in the stomatal limitation to photosynthesis (Fig. 3d) with increasing age. When combined with the absence of an age-related change in $V_{c,\text{max}}$ or J_{max} , these results suggest that the shape of $A:c_i$ curve did not vary with age, but rather that older trees were operating at a lower c_i (Fig. 4). In addition, needle SLA declined significantly with age (Fig. 5) from 47 to 40 $\text{cm}^2 \text{ g}^{-1}$ at 15 and 115 years of age, respectively, and the ratio of SA to leaf area increased with age from 3.1 $\text{cm}^2 \text{ m}^{-2}$

Age (years)	Pine foliage		Pine litter		% Pine N retrans location
	N (%)	C (%)	N (%)	C (%)	
14	1.14 (0.05)	51.3 (0.5)	0.49 (0.02)	52.7 (0.1)	57 (0.1)
19	1.19 (0.04)	51.3 (0.4)	0.69 (0.06)	52.0 (0.2)	42 (0.1)
22	1.21 (0.03)	52.1 (0.4)	0.59 (0.03)	50.6 (0.2)	51 (0.1)
36	1.24 (0.05)	52.1 (0.2)	0.55 (0.02)	51.6 (0.2)	56 (0.1)
42	NA	NA	0.46 (0.01)	52.6 (0.2)	NA
53	1.23 (0.06)	52.5 (0.4)	0.54 (0.02)	52.7 (0.2)	56 (0.1)
71	1.15 (0.04)	51.4 (0.3)	0.47 (0.02)	52.1 (0.2)	59 (0.1)
74	NA	NA	0.59 (0.02)	50.5 (0.3)	NA
79	1.16 (0.05)	50.9 (0.5)	0.50 (0.02)	50.6 (0.2)	57 (0.1)
97	NA	NA	0.48 (0.02)	52.4 (0.2)	NA
114	1.31 (0.05)	52.4 (0.5)	0.45 (0.02)	52.3 (0.2)	66 (0.1)
115	1.13 (0.04)	51.3 (0.4)	0.65 (0.02)	51.2 (0.2)	43 (0.1)

Values are the mean (± 1 SE) of 8–10 replicates. The collection of fresh foliage by shotgun was not feasible in three of the 12 sites; these values are indicated as NA. There were no significant relationships between measured variables and stand age (regression, $P > 0.05$).

at age 15 to $\sim 6 \text{ cm}^2 \text{ m}^{-2}$ at age 115 (Table 1; $y = 4.01 + 0.017x$, $P < 0.05$, $r^2 = 0.34$).

The decline in c_i with increasing age was corroborated by wood C isotopic measurements, which reflect

time-integrated c/c . values (Katul *et al.* 2000). Wood $\delta^{13}\text{C}$ increased from $-27.8\text{\textperthousand}$ at age 14 to $-26.3\text{\textperthousand}$ at age 115 ($y = -27.29 + 0.0125x$, $r^2 = 0.45$, $P < 0.05$), which represents a decline in c/c . from 0.65 to 0.58 ($y = 0.65 - 0.0005x$, $r^2 = 0.45$,

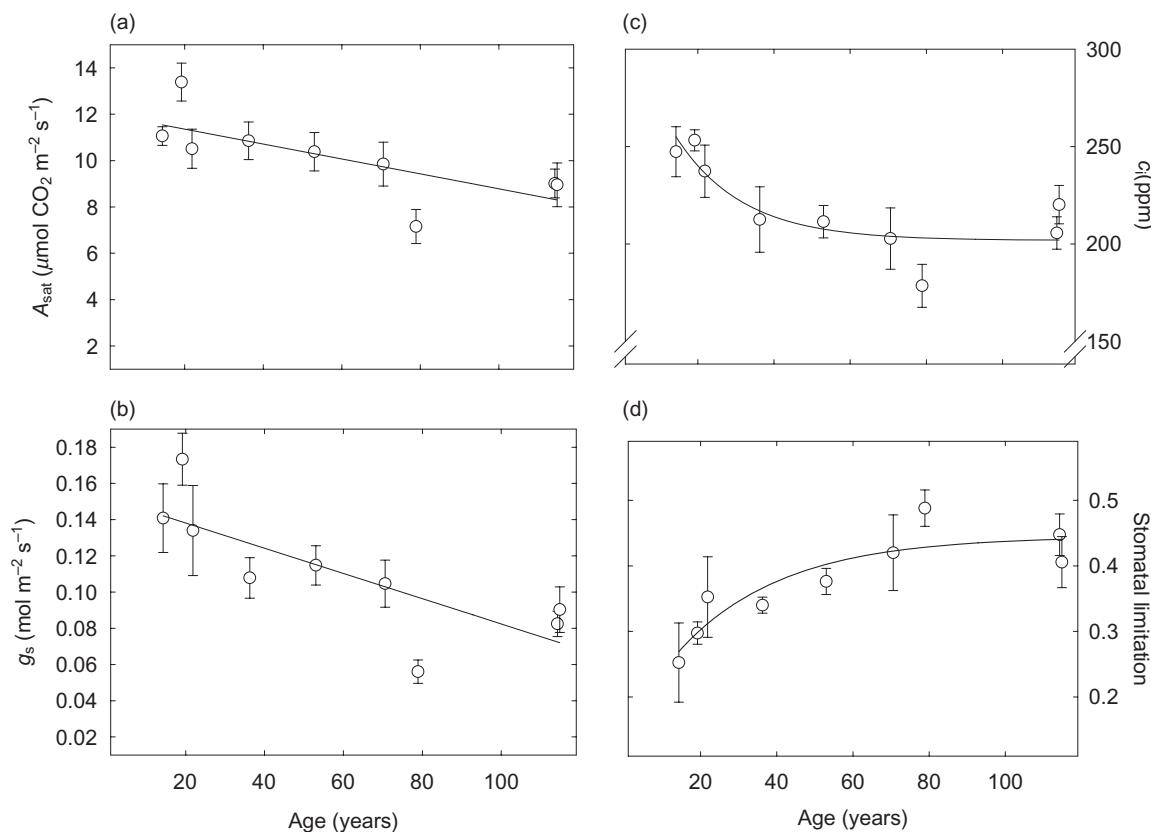


Figure 3. Gas exchange parameters of loblolly pine (*Pinus taeda*) needles at light saturation and atmospheric $[\text{CO}_2]$ in relation to stand age. Light-saturated photosynthetic CO_2 uptake (A_{sat}) declined with increasing age (a): $y = 12.0 - 0.032x$, $P < 0.05$, $r^2 = 0.47$. Stomatal conductance to H_2O (g_s) declined with increasing age (b): $y = 0.15 - 0.0007x$, $P < 0.05$, $r^2 = 0.56$. The concentration of CO_2 within needle air-spaces (c_i ; c) declined in a negative exponential manner with increasing age: $y = 201.8 + 120.8 * \exp(-0.057x)$, $P < 0.05$, $r^2 = 0.65$. Stomatal limitation to the realized rate of photosynthesis (d) increased in a saturating exponential manner with increasing age: $y = 0.15 + 0.296 * \exp(-0.366x)$, $P < 0.01$, $r^2 = 0.75$.

Table 2. Foliar N and C concentrations, nitrogen retranslocation and the ratio of sapwood area (SA) to leaf area of *Pinus taeda* at 12 forests of varying age in North Carolina

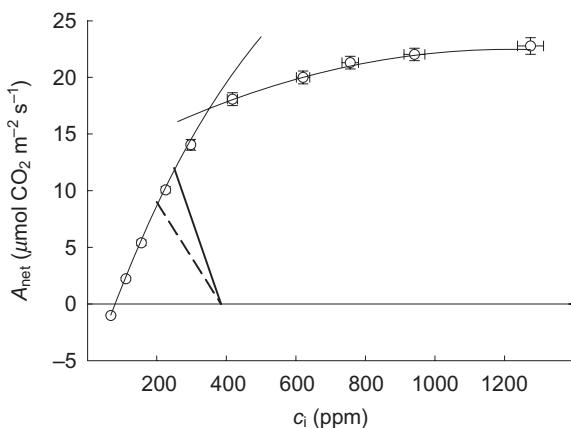


Figure 4. Net CO_2 assimilation (A_{net}) in relation to the concentration of CO_2 within needle air-spaces (c_i), reflecting an average of 90 $A:c_i$ curves within nine stands of loblolly pine forests (*Pinus taeda*) of varying age. The shape of the curve did not vary with age. Error bars are $\pm 1 \text{ SE}$. The lines are the CO_2 supply curves that connect atmospheric $[\text{CO}_2]$ to c_i ; the slopes of these lines are proportional to stomatal conductance. The solid line reflects the youngest forests, while the dashed line reflects the oldest forests.

$P < 0.05$). Estimates of mean c_i/c_c from gas exchange and wood $\delta^{13}\text{C}$ were correlated [isotopic $c_i/c_c = 0.321 * (\text{gas exchange } c_i/c_c) + 0.442$, $r^2 = 0.39$, $P < 0.05$].

Sap-flow measurements indicated that day-time leaf transpiration rates declined by ~60% with increasing age (Fig. 6a). The youngest forests had the highest day-time canopy-weighted stomatal conductance (G_c) at low VPD, but these forests rapidly reduced G_c as VPD increased (Fig. 6b). In contrast, the oldest forests always had low G_c , and were thus less sensitive to increases in VPD. Age-related differences in G_c were most pronounced at low VPD values.

The dendroclimatological analysis demonstrated that radial growth of all forest ages was greatest in years with

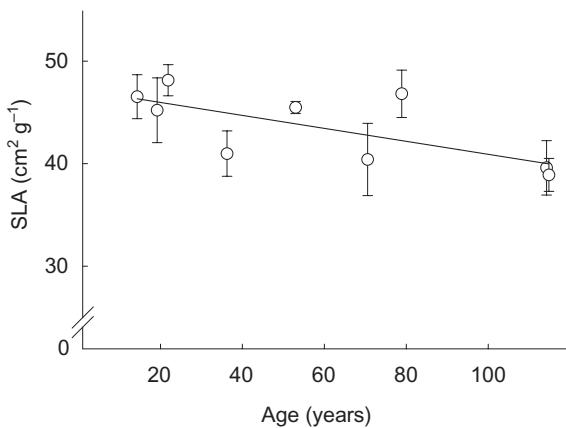


Figure 5. Specific leaf area (SLA) of loblolly pine (*Pinus taeda*) needles in relation to stand age. All error bars reflect $\pm 1 \text{ SE}$ of 8–10 measurements per site. SLA declined with increasing age: $y = 47.2 - 0.063x$, $r^2 = 0.41$, $P < 0.05$.

cool summers and high water availability (i.e. high values of PDSI; Fig. 7). However, the strength of the correlation between inter-annual variation in climate and radial growth declines with age. Thus, the youngest pines were more sensitive to hot and dry summers relative to older forests. Summer mean temperature and PDSI were not correlated ($P > 0.5$).

DISCUSSION

Light-saturated photosynthesis declined by ~25% with increasing age, and the corresponding decline in stomatal conductance and decrease in c_i suggest that this was driven by increasing hydraulic limitation. Results were not consistent with any of the predictions regarding the hypothesis of increasing N limitation, while all but one of the predictions regarding the hypothesis of increasing hydraulic limitation were supported. Leaf- and canopy-level stomatal conductance declined, c_i/c_c declined, stomatal limitation increased,

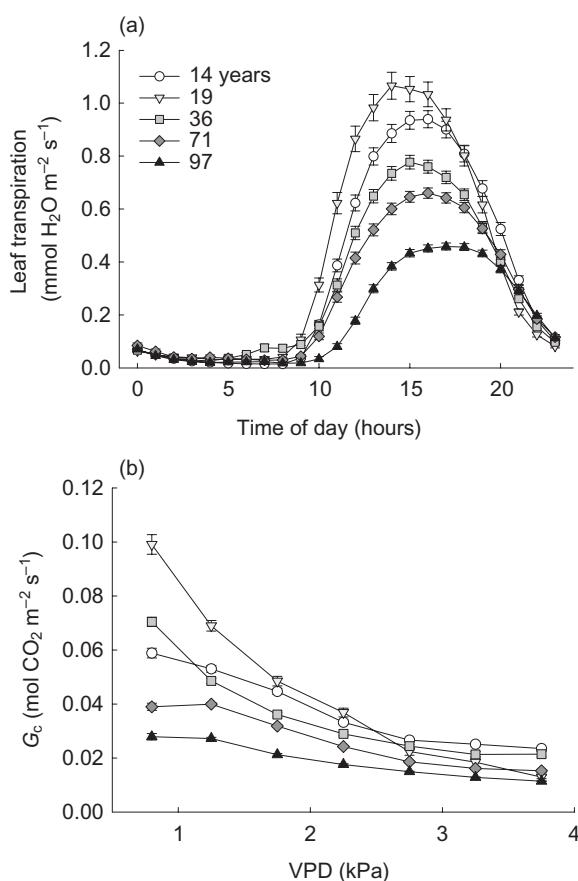


Figure 6. Sap-flow-derived leaf transpiration over time (E ; a) and canopy-weighted stomatal conductance to CO_2 (G_c) in response to vapour pressure deficit (VPD; b) in loblolly pine (*Pinus taeda*) trees of varying age. Values reflect the mean of measurements from 12 May and 1 October 2008, and error bars reflect $\pm 1 \text{ SE}$ of 3–520 measurements per point (very high VPD values were rare). Only day-time values from 10 to 20 h were used to compute the G_c averages.

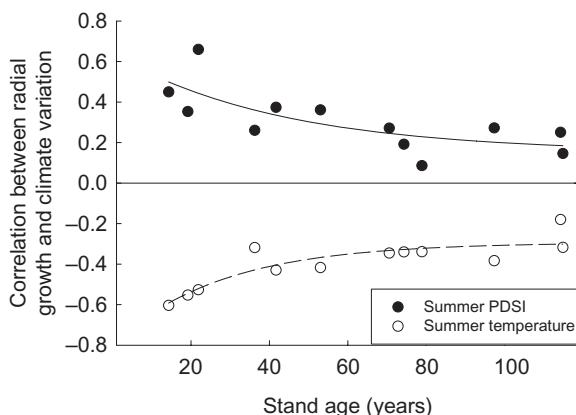


Figure 7. Dendroclimatological analysis of radial growth by loblolly pine (*Pinus taeda*) forests of varying age. Radial growth was positively correlated with the Palmer drought severity index (PDSI) and negatively related to temperature in all sites, but the strength of these correlations declined with age. PDSI: $y = 0.15 + 0.49 * \exp(0.023x)$, $r^2 = 0.46$, $P < 0.05$; temperature: $y = -0.79 + 0.5 * \exp(-0.036x)$, $r^2 = 0.71$, $P < 0.01$. Summer was defined as June–August.

SLA decreased and the ratio of SA to leaf area increased. We conclude that hydraulic limitation increasingly limits photosynthesis in ageing loblolly pines. The decreasing sensitivity of radial growth to adverse inter-annual climate variation with increasing age, the opposite of our prediction, suggests that hydraulic limitation may reflect an increasingly conservative growth strategy of pine trees as they age.

The hydraulic limitation hypothesis has broad support for a variety of forest types. Delzon *et al.* (2005) observed that photosynthetic capacity of maritime pine (*Pinus pinaster*) did not vary with age where NPP declined, and suggested that reduced stomatal conductance caused the decrease in GPP and hence NPP. A number of sap-flow investigations have demonstrated reduced water use by trees of increasing age (Ryan *et al.* 2000; Zimmerman *et al.* 2000; Roberts, Vertessy & Grayson 2001; Köstner, Falge & Tenhunen 2002; Phillips *et al.* 2003; Moore *et al.* 2004; Delzon & Loustau 2005), which have been corroborated by leaf-level gas exchange studies demonstrating reduced g_s and A_{net} in old trees (Yoder *et al.* 1994; Koch *et al.* 2004; Greenwood *et al.* 2008). However, the only study to link whole-forest C budgets with a test of the hydraulic limitation hypothesis did not find a change in conductance of sufficient magnitude to explain the observed decline in GPP (Barnard & Ryan 2003; Ryan *et al.* 2004). Thus, many old and tall trees show a consistent pattern of increasing hydraulic limitation, but it is unclear if this can generally explain the age-related decline in NPP across forest types.

The data presented here support five predictions from the hydraulic limitation hypothesis specified by Ryan *et al.* (2006): (1) reduced canopy G_s ; (2) reduced leaf level g_s ; (3) increased $\delta^{13}\text{C}$ and reduced c/c_s ; and (4) reduced A_{sat} . Additionally, (5) there was evidence of compensation for increasing hydraulic limitation, as the ratio of SA to leaf area ($\text{cm}^2 \text{m}^{-2}$) increased significantly with age; SA to leaf

area was $3.1 \text{ cm}^2 \text{ m}^{-2}$ at age 14, and $6 \text{ cm}^2 \text{ m}^{-2}$ at age 115. DeLucia, Maherli & Carey (2000) demonstrated that published values of pine SA to leaf area were positively related to summer average daily maximum VPD; the youngest forest measured here corresponds to pines growing at a VPD of $\sim 1.5 \text{ kPa}$, while the oldest forests correspond to pines growing at $\sim 2.8 \text{ kPa}$. Thus, pines are known to vary the amount of sapwood per unit leaf area in response to evaporative demand, and pines observed here compensated across the full range of observed values in the DeLucia *et al.* (2000) data set.

The reduction in SLA with increasing age has been observed previously and attributed to a reduction in turgor-driven cellular expansion (Koch *et al.* 2004; Woodruff, Bond & Meinzer 2004). A reduction in SLA with increasing height in the canopy of individual forests is commonly observed and often attributed to the physiological differences between sun and shade foliage (Harley, Guenther & Zimmerman 1996; Koch *et al.* 2004; Ambrose, Sillett & Dawson 2009; Mullin *et al.* 2009). However, all of the needles measured here were sun leaves from the top of forest canopies, implicating a physiological difference based on height such as reduced turgor, not physiological acclimations to light availability.

While the dendroclimatological results (Fig. 7) were the opposite of predicted, they were consistent with the G_c measurements derived from sap-flow (Fig. 6b). G_c of the youngest trees was very sensitive to variation in evaporative demand, while the oldest trees had consistently low G_c that varied little with VPD. This is consistent with the correlations between radial growth and inter-annual climate variation (Fig. 7); growth by the youngest trees was most sensitive to environmental variation related to water availability, while growth by the oldest trees was less affected. Together, these results suggest that these trees increasingly adopt a stress-tolerant, conservative growth strategy with increasing age. Grime (1977) hypothesized that forest communities should change from ruderal to stress-tolerating species during the course of secondary succession. The same process of growth strategy acclimation may occur within individual species exhibiting state-dependent plasticity in life history strategies, as has been recognized in animals (McNamara & Houston 1996).

The results of this study are in general agreement with the literature, showing little support for increasing N limitation as a cause of the age-related decline in GPP, and hence NPP. While N limitation of NPP is widespread across ecosystem types and particularly strong in temperate forests (Elser *et al.* 2007; LeBauer & Treseder 2008), there is no established pattern of declining nutrient availability with increasing age commensurate with declining NPP (Ryan *et al.* 1997a; Olsson, Binkley & Smith 1998). Some ecosystem modelling efforts have suggested that nutrient availability should decline with age as N is immobilized in woody biomass (Murty & McMurtrie 2000), but observational support for this process is rare (Johnson 2006), and N availability can even increase as forests age (Smithwick *et al.* 2009).

In conclusion, leaf-level photosynthetic rates by loblolly pine declined with increasing age through increasing hydraulic limitation of stomatal conductance, not increasing nutrient limitation. Combined with previous measures of declining production in these forests (Drake *et al.* 2010), it appears that hydraulic limitation of GPP can explain the observed age-related decline in NPP for these loblolly pines. Thus, this study supports the hypothesis of Ryan *et al.* (1997a) that GPP is the driver of age-related changes in NPP, rather than R_a as was previously thought (e.g. Odum 1969). This conclusion has implications for the future of the C sink in aggrading temperate forests (Pacala *et al.* 2001), because elements of global change that primarily affect photosynthesis (i.e. CO₂) may have a larger influence on the future productivity of these forests than elements primarily affecting R_a (i.e. temperature).

ACKNOWLEDGMENTS

We gratefully acknowledge George Hendrey and Robert Nettles (Brookhaven National Laboratory) for the operation of the Duke FACE experiment. We thank Judson Edeburn and Marissa Hartzler (Duke Forest) for assistance identifying the chronosequence stands, providing historical management information and allowing the use of shotguns on University property. Richard Thomas, Andrew Leakey and Patrick Morgan gave helpful suggestions concerning the gas exchange measurements. We thank Ram Oren and Eric Ward for help designing and implementing the sap-flow instrumentation. The Duke FACE experiment was supported by the Office of Science (BER), US Department of Energy Grant No. DE-FG02-95ER62083 and through its Southeast Regional Center (SERC) of the National Institute for Global Environmental Change (NIGEC) under Cooperative Agreement No. DE-FC02-03ER63613. Additional support was provided by DOE (BER) Grant No. DE-FG02-04ERG384. J.D. gratefully acknowledges support through a University Distinguished Fellowship from the University of Illinois, and multiple travel grants from the Department of Plant Biology and the Program of Ecology, Evolution, and Conservation Biology at the University of Illinois.

REFERENCES

- Abramoff M.D., Magelhaes P.J. & Ram S.J. (2004) Image processing with ImageJ. *Biophotonics International* **11**, 36–42.
- Ambrose A.R., Sillett S.C. & Dawson T.E. (2009) Effects of tree height on branch hydraulics, leaf structure and gas exchange in California redwoods. *Plant, Cell & Environment* **32**, 743–757.
- Barnard H.R. & Ryan M.G. (2003) A test of the hydraulic limitation hypothesis in fast-growing *Eucalyptus saligna*. *Plant, Cell & Environment* **26**, 1235–1245.
- Bernacchi C.J., Portis A.R., Nakano H., von Caemmerer S. & Long S.P. (2002) Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis *in vivo*. *Plant Physiology* **130**, 1992–1998.
- Bernacchi C.J., Pimentel C. & Long S.P. (2003) *In vivo* temperature response functions for parameters required to model RuBP-limited photosynthesis. *Plant, Cell & Environment* **26**, 1419–1430.
- Bunn A.G. (2008) A dendrochronology program library in R (dpIR). *Dendrochronologia* **26**, 115–124.
- Chen J.M. (1996) Optically-based methods for measuring seasonal variation of leaf area index in boreal conifer stands. *Agricultural and Forest Meteorology* **80**, 135–163.
- Crous K.Y. & Ellsworth D.S. (2004) Canopy position affects photosynthetic adjustments to long-term elevated CO₂ concentration (FACE) in aging needles in a mature *Pinus taeda* forest. *Tree Physiology* **24**, 961–970.
- Dawson T.E., Mambelli S., Plamboeck A.H., Templer P.H. & Tu K.P. (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics* **33**, 507–559.
- DeLucia E.H., Maherli H. & Carey E.V. (2000) Climate-driven changes in biomass allocation in pines. *Global Change Biology* **6**, 587–593.
- DeLucia E.H., Drake J.E., Thomas R.B. & Gonzalez-Meler M. (2007) Forest carbon use efficiency: is respiration a constant fraction of gross primary production? *Global Change Biology* **13**, 1157–1167.
- Delzon S. & Loustau D. (2005) Age-related decline in stand water use: sap flow and transpiration in a pine forest chronosequence. *Agricultural and Forest Meteorology* **129**, 105–119.
- Delzon S., Bosc A., Cantet L. & Loustau D. (2005) Variation of the photosynthetic capacity across a chronosequence of maritime pine correlates with needle phosphorus concentration. *Annals of Forest Science* **62**, 537–543.
- Denman K.L., Brasseur G., Chidthaisong A., *et al.* (2007) Couplings between changes in the climate system and biogeochemistry. In *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor & H.L. Miller) pp. 499–587. Cambridge University Press, Cambridge, UK and New York, NY, USA.
- Drake J.E. (2010) Environmental and developmental regulation of carbon cycling in a warm temperature forest. *Doctoral dissertation*, University of Illinois, Urbana, IL USA, p. 183.
- Elser J.J., Bracken M.E.S., Cleland E.E., Gruner D.S., Harpole W.S., Hillebrand H., Ngai J.T., Seabloom E.W., Shurin J.B. & Smith J.E. (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**, 1135–1142.
- Ewers B.E. & Oren R. (2000) Analyses of assumptions and errors in the calculations of stomatal conductance from sap flux measurements. *Tree Physiology* **20**, 579–589.
- Ewers B.E., Oren R., Johnsen K.H. & Landsberg J.J. (2001) Estimating maximum mean canopy stomatal conductance for use in models. *Canadian Journal of Forest Research* **31**, 198–207.
- Farquhar G.D., Oleary M.H. & Berry J.A. (1982) On the relationship between carbon isotope discrimination and the intercellular carbon-dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9**, 121–137.
- Field C.B., Behrenfeld M.J., Randerson J.T. & Falkowski P. (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. *Science* **281**, 237–240.
- Finzi A.C., Allen A.S., DeLucia E.H., Ellsworth D.S. & Schlesinger W.H. (2001) Forest litter production, chemistry, and decomposition following two years of free-air CO₂ enrichment. *Ecology* **82**, 470–484.
- Franco B. & Kishor W. (2004) DENDROCLIM2002: a C++ program for statistical calibration of climate signals in tree-ring chronologies. *Computers & Geosciences* **30**, 303–311.
- Gower S.T., McMurtrie R.E. & Murty D. (1996) Aboveground net primary production decline with stand age: potential causes. *Trends in Ecology & Evolution* **11**, 378–382.

- Granier A. (1987) Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. *Tree Physiology* **3**, 309–320.
- Greenway K.J., Macdonald S.E. & Lieffers V.J. (1992) Is long-lived foliage in *Picea mariana* an adaptation to nutrient-poor conditions? *Oecologia* **91**, 184–191.
- Greenwood M.S., Ward M.H., Day M.E., Adams S.L. & Bond B.J. (2008) Age-related trends in red spruce foliar plasticity in relation to declining productivity. *Tree Physiology* **28**, 225–232.
- Grime J.P. (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist* **111**, 1169–1194.
- Hamilton J.G., Delucia E.H., George K., Naidu S.L., Finzi A.C. & Schlesinger W.H. (2002) Forest carbon balance under elevated CO₂. *Oecologia* **131**, 250–260.
- Harley P., Guenther A. & Zimmerman P. (1996) Effects of light, temperature, and canopy position on net photosynthesis and isoprene emission from sweetgum leaves. *Tree Physiology* **16**, 25–32.
- Harrington R.A. & Fownes J.H. (1995) Radiation interception and growth of planted and coppice stands of four fast-growing tropical trees. *Journal of Applied Ecology* **32**, 1–8.
- Huston M.A. & Wolverton S. (2009) The global distribution of net primary production: resolving the paradox. *Ecological Monographs* **79**, 343–377.
- Johnson D.W. (2006) Progressive N limitation in forests: review and implications for long-term responses to elevated CO₂. *Ecology* **87**, 64–75.
- Katul G.G., Ellsworth D.S. & Lai C.T. (2000) Modeling assimilation and intercellular CO₂ from measured conductance: a synthesis of approaches. *Plant, Cell & Environment* **23**, 1313–1328.
- Kira T. & Shidei T. (1967) Primary production and turnover of organic matter in different forest ecosystems of the Western Pacific. *Japanese Journal of Ecology* **17**, 70–87.
- Koch G.W., Sillett S.C., Jennings G.E. & Davis S.D. (2004) The limits to tree height. *Nature* **428**, 851–854.
- Köstner B., Falge E. & Tenhunen J.D. (2002) Age-related effects on leaf area/sapwood area relationships, canopy transpiration and carbon gain of Norway spruce stands (*Picea abies*) in the Fichtelgebirge, Germany. *Tree Physiology* **22**, 567–574.
- LeBauer D.S. & Treseder K.K. (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**, 371–379.
- Liu X., Ellsworth D.S. & Tyree M.T. (1997) Leaf nutrition and photosynthetic performance of sugar maple (*Acer saccharum*) in stands with contrasting health conditions. *Tree Physiology* **17**, 169–178.
- Long S.P. & Bernacchi C.J. (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany* **54**, 2393–2401.
- Luo Y., Currie W.S., Dukes J.S., et al. (2004) Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. *Bioscience* **54**, 731–739.
- McCarthy H.R., Oren R., Johnsen K.H., Gallet-Budynek A., Pritchard S.G., Cook C.W., LaDeau S.L., Jackson R.B. & Finzi A.C. (2009) Re-assessment of plant carbon dynamics at the Duke free-air CO₂ enrichment site: interactions of atmospheric [CO₂] with nitrogen and water availability over stand development. *New Phytologist* **185**, 514–528.
- McNamara J.M. & Houston A.I. (1996) State-dependent life histories. *Nature* **380**, 215–221.
- Moller C.M., Muller D. & Nielsen J. (1954) Graphic presentation of dry matter production in European beech. *Det Forstlige Forsøgs-vasen I Danmark* **21**, 327–335.
- Moore G.W., Bond B.J., Jones J.A., Phillips N. & Meinzer F.C. (2004) Structural and compositional controls on transpiration in 40- and 45-year-old riparian forests in Western Oregon, USA. *Tree Physiology* **24**, 481–491.
- Mullin L.P., Sillett S.C., Kock G.W., Tu K.P. & Antoine M.E. (2009) Physiological consequences of height-related morphological variation in *Sequoia sempervirens* foliage. *Tree Physiology* **29**, 999–1010.
- Murty D. & McMurtrie R.E. (2000) The decline of forest productivity as stands age: a model-based methods for analyzing causes for the decline. *Ecological Modeling* **134**, 185–205.
- Murty D., McMurtrie R.E. & Ryan M.G. (1996) Declining forest productivity in ageing forest stands – a modeling analysis of alternative hypotheses. *Tree Physiology* **16**, 187–200.
- Naidu S.L., DeLucia E.H. & Thomas R.B. (1998) Contrasting patterns of biomass allocation in dominant and suppressed loblolly pine. *Canadian Journal of Forest Research* **28**, 1116–1124.
- Odum E.P. (1969) Strategy of ecosystem development. *Science* **29**, 234–238.
- Ollinger S.V., Aber J.D. & Federer C.A. (1998) Estimating regional forest productivity and water yield using an ecosystem model linked to a GIS. *Landscape Ecology* **13**, 323–334.
- Olsson U., Binkley D. & Smith F.W. (1998) Nitrogen supply, nitrogen use, and production in an age sequence of lodgepole pine. *Forest Science* **44**, 454–457.
- Pacala S.W., Hurtt G.C., Baker D., et al. (2001) Consistent land- and atmosphere-based US carbon sink estimates. *Science* **292**, 2316–2320.
- Phillips N., Oren R. & Zimmerman R. (1996) Radial patterns of xylem sap flow in non-, diffuse- and ring-porous tree species. *Plant, Cell & Environment* **19**, 983–990.
- Phillips N., Bond B.J., McDowell N.G., Ryan M.G. & Schauer A. (2003) Leaf area compounds height-related hydraulic costs of water transport in Oregon white oak trees. *Functional Ecology* **17**, 832–840.
- Pregitzer K.S. & Euskirchen E.S. (2004) Carbon cycling and storage in world forests: biome patterns related to forests age. *Global Change Biology* **10**, 2052–2077.
- R Development Core Team (2007) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Richardson A.D., Hollinger D.Y., Aber J.D., Ollinger S.V. & Braswell B.H. (2007) Environmental variation is directly responsible for short- but not long-term variation in forest-atmosphere carbon exchange. *Global Change Biology* **13**, 788–803.
- Roberts S., Vertessy R. & Grayson R. (2001) Transpiration from *Eucalyptus sieberi* (L. Johnson) forests of different age. *Forest Ecology and Management* **143**, 153–161.
- Ryan M.G. & Waring R.H. (1992) Maintenance respiration and stand development in a sub-alpine lodgepole pine forest. *Ecology* **73**, 2100–2108.
- Ryan M.G. & Yoder B.J. (1997) Hydraulic limits to tree height and growth. *Bioscience* **47**, 235–242.
- Ryan M.G., Hubbard R.M., Pongracic S., Raison R.J. & McMurtrie R.E. (1996) Foliation, fine-root, woody-tissue and stand respiration in *Pinus radiata* in relation to nutrient status. *Tree Physiology* **16**, 333–343.
- Ryan M.G., Binkley D. & Fownes J.H. (1997a) Age-related decline in forest productivity: pattern and process. *Advances in Ecological Research* **27**, 213–262.
- Ryan M.G., Lavigne M.B. & Gower S.T. (1997b) Annual carbon cost of autotrophic respiration in boreal forest ecosystems in relation to species and climate. *Journal of Geophysical Research* **102**, 333–343.
- Ryan M.G., Bond B.J., Law B.E., Hubbard R.M., Woodruff D.,

- Cienciala E. & Kucera J. (2000) Transpiration and whole-tree conductance in ponderosa pine trees of different heights. *Oecologia* **124**, 553–560.
- Ryan M.G., Binkley D., Fownes J.H., Giardina C.P. & Senock R.S. (2004) An experimental test of the causes of forest growth decline with stand age. *Ecological Monographs* **74**, 393–414.
- Ryan M.G., Phillips N. & Bond B.J. (2006) The hydraulic limitation hypothesis revisited. *Plant, Cell & Environment* **29**, 367–381.
- Schäfer K.V.R., Oren R., Lai C.T. & Katul G.G. (2002) Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO₂ concentration. *Global Change Biology* **8**, 895–911.
- Smithwick E.A.H., Kashian D.M., Ryan M.G. & Turner M.G. (2009) Long-term ecosystem nitrogen storage and soil nitrogen availability in post-fire lodgepole pine ecosystems. *Ecosystems* **12**, 792–806.
- Thomas V., Treitz P., McCaughey J.H., Noland T. & Rich L. (2008) Canopy chlorophyll concentration estimation using hyperspectral and lidar data for a boreal mixedwood forest in Northern Ontario, Canada. *International Journal of Remote Sensing* **29**, 1029–1052.
- Whittaker R.H. & Woodwell G.M. (1967) Surface area relations of woody plants and forest communities. *American Journal of Botany* **54**, 931–939.
- Woodruff D.R., Bond B.J. & Meinzer F.C. (2004) Does turgor limit growth in tall trees? *Plant, Cell & Environment* **27**, 229–236.
- Wullschleger S.D. & King A.W. (2000) Radial variation in sap velocity as a function of stem diameter and sapwood thickness in yellow-poplar trees. *Tree Physiology* **20**, 511–518.
- Yoda K., Shinozaki K., Ogawa H., Hozumi K. & Kira T. (1965) Estimation of the total amount of respiration in woody organs of trees and forest communities. *Journal of Biology, Osaka City University* **16**, 15–26.
- Yoder B.J., Ryan M.G., Waring H., Schoettle A.W. & Kaufmann M.R. (1994) Evidence of reduced photosynthetic rates in old trees. *Forest Science* **40**, 513–527.
- Zhang S. & Allen H.L. (1996) Foliar nutrient dynamics of 11-year-old loblolly pine (*Pinus taeda*) following nitrogen fertilization. *Canadian Journal of Forest Research* **26**, 1426–1439.
- Zimmerman R., Schulze E.D., Wirth C., Schulze E.E., McDonald K.C., Vygodskaya N.N. & Ziegler W. (2000) Canopy transpiration in a chronosequence of Central Siberian pine forests. *Global Change Biology* **6**, 25–37.

Received 5 March 2010; received in revised form 12 May 2010; accepted for publication 13 May 2010