

# Mechanisms of age-related changes in forest production: the influence of physiological and successional changes

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## Abstract

Net primary production (*NPP*) declines as forests age, but the causal role of decreased gross primary production (*GPP*), or increased autotrophic respiration ( $R_a$ ) is still a matter of debate. This uncertainty complicates predicted responses to future climate, as higher atmospheric carbon dioxide ( $CO_2$ ) concentrations may amplify the carbon (C)-sink in temperate forests if *GPP* controls the decline in *NPP*, but increased temperatures may decrease this C-sink if  $R_a$  controls the *NPP* decline. We quantified *NPP* in forests dominated by loblolly pine (*Pinus taeda*) in North Carolina, USA that varied from 14 to 115 years old. We used a sap-flow approach to quantify summer canopy photosynthesis by pines and later-successional hardwood trees, and measured wood  $CO_2$  efflux to investigate age-related changes in pine  $R_a$ . Despite increasing production by later-successional hardwoods, an 80% decline in pine *NPP* caused ecosystem *NPP* to decline with age by ~40%. The decline in pine *NPP* was explained by reduced stomatal conductance and photosynthesis, supporting the hypothesis that increasing hydraulic limitation and declining *GPP* drove the age-related decline of *NPP* in this species. The difference between *GPP* and *NPP* indicated that pine  $R_a$  also declined with age; this was corroborated by measurements of reduced stem  $CO_2$  efflux with increasing age. These results indicate that C cycling in these successional temperate forests is controlled by C input from *GPP*, and elements of global change that increase *GPP* may increase the C-sink in aging warm-temperate pine forests.

**Keywords:** autotrophic respiration, *GPP*, Granier, gross primary production, hydraulic limitation hypothesis, net primary production, *NPP*,  $R_a$ , sap-flow

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## Introduction

Predictions of future climate by coupled biosphere-atmosphere models are highly sensitive to assumptions regarding the responsiveness of terrestrial carbon (C) cycling to atmospheric carbon dioxide ( $CO_2$ ), and temperature (Dufresne *et al.*, 2002; Friedlingstein *et al.*, 2006; Meehl *et al.*, 2007). Respiration is highly sensitive to increases in temperature, but only indirectly related to increases in atmospheric  $CO_2$  through its affect on growth (Davey *et al.*, 2004; Leakey *et al.*, 2009). In contrast, photosynthesis is highly sensitive to increases in atmospheric  $CO_2$  (Ainsworth & Long, 2005) but less sensitive to temperature than respiration (Atwell *et al.*, 1999; Atkin & Tjoelker, 2003; Campbell *et al.*, 2007). Thus, the net response of ecosystem C cycling to the simultaneous increase in atmospheric  $CO_2$  and tem-

perature expected in the future (Meehl *et al.*, 2007) depends on whether respiration or photosynthesis is the primary mechanism controlling C cycling in terrestrial ecosystems.

Aggrading temperate forests have been identified as an important sink for atmospheric  $CO_2$  (Pacala *et al.*, 2001; Albani *et al.*, 2006), but the future of this sink is uncertain as most aging forests undergo a decline in net primary production (*NPP*; reviews by Ryan *et al.*, 1997a; Pregitzer & Euskirchen, 2004; DeLucia *et al.*, 2007). Two physiological hypotheses have been put forward to explain the age-related decline in *NPP*. The earliest hypothesis was that increasing autotrophic respiration ( $R_a$ ) from accumulating woody biomass reduced the fraction of gross primary productivity (*GPP*) available for *NPP* (Möller *et al.*, 1954; Yoda *et al.*, 1965; Kira & Shidea, 1967; Whittaker & Woodwell, 1967; Odum, 1969). While one literature synthesis partially supports this claim (DeLucia *et al.*, 2007), most recent research has been unable to detect an increase in  $R_a$  of sufficient magnitude to explain the decline in *NPP* (Ryan & Waring, 1992; Harrington & Fownes, 1995; Ryan *et al.*,

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1996, 1997b, 2004). These observations lead to the second hypothesis; *NPP* declines because *GPP* declines in aging forests. The decline in *GPP* is presumably caused by reduced photosynthetic rates in older trees (Yoder *et al.*, 1994; Hubbard *et al.*, 1999) because of increasing hydraulic (Ryan & Yoder, 1997; Ryan *et al.*, 2006) or nutrient limitations (Gower *et al.*, 1996). It is important to determine whether  $R_a$  or *GPP* controls the age-related decline in *NPP* to predict the effect of global change on the C sink in aging forests.

Despite the relevance of this research to global change and its relation to foundational principles in ecosystem ecology (Odum, 1969), there have been few direct tests of the mechanism causing the age-related decline in *NPP*. Only one study has directly measured *NPP* and  $R_a$  in a forest undergoing a decline in *NPP* (*GPP* was estimated by the sum of *NPP* and  $R_a$ ; Ryan *et al.*, 2004). This study indicated that a decline in *GPP* and a reallocation of C belowground explained the decline in *NPP* in aging *Eucalyptus saligna* plantations, but the trees were <6 years old and natural colonization by other species was experimentally suppressed. Thus, it is unclear whether these results can be generalized to other forests that undergo succession over longer time-scales as the dominant trees age and *NPP* declines.

We quantified *NPP* across a chronosequence of forests varying from 14 to 115 years old that exhibited substantial successional change in community composition. Previously, we demonstrated that photosynthetic rates by the dominant pines decline with age because of increasing hydraulic limitation (Drake *et al.*, 2010). Here, we directly quantified the age-related change in *GPP* to test the hypothesis that increasing hydraulic limitation reduces *GPP* and thus *NPP* in these aging forests.

## Methods

### Study sites

This research was performed in East-central North Carolina, USA. Forests in this region undergo a robust pattern of successional change, as early-successional *Pinus* spp. are replaced by later-successional species such as *Liquidambar styraciflua*, *Liriodendron tulipifera*, *Ulmus elata*, *Acer rubrum*, *Quercus* spp., and *Carya* spp. (Billings, 1938; McQuilkin, 1940; Oosting, 1942; Bormann, 1953; McDonald *et al.*, 2007).

Twelve stands were identified within the Korstian and Durham divisions of the Duke Forest, located in Durham County, NC, USA. 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 loblolly pines (*Pinus taeda* L.) varied from 14 to 115 years, including the oldest loblolly pines present in this region. The age range of the forests included in this study capture two

major processes that may impact ecosystem development (1) potential age-related changes in pine physiology and (2) changing community composition. The maximum distance between stands was 6 km. All soils were heavily weathered, clay-rich Alfisol soils of the Helena series with relatively low nitrogen and phosphorus availability (Schlesinger & Lichter, 2001). Site index, a proxy for soil quality (Avery & Burkhart, 1983), was narrowly constrained between 84 and 86 for loblolly pine, indicating that soil conditions were similar among sites. The Helena soil series was chosen for site selection because it closely matches the soil at the nearby Duke free air CO<sub>2</sub> enrichment (FACE) site (Hamilton *et al.*, 2002), enabling comparisons between data presented here and the extensively measured, 26-year-old forest at the FACE site, ~5 km to the West. These narrow site selection criteria were implemented to isolate the effect of age from other factors that may cause differences between stands, as natural variation in forest composition and function can be large (Bradford *et al.*, 2008).

A single large plot was established in each of the 12 forest stands and all stems >4 cm diameter at breast height (dbh, 1.4 m) were surveyed. Plot size was adjusted to include a minimum of 150 trees as suggested by Harmon *et al.* (2007), so plot size increased as stem density decreased; all plots were 1000–2000 m<sup>2</sup>.

### NPP

*NPP* is theoretically defined as the difference between *GPP* and  $R_a$  (Chapin *et al.*, 2006). In practice, *NPP* is measured by the sum of biomass production components (Clark *et al.*, 2001) because of the difficulties in measuring *GPP* and  $R_a$  directly. We operationally defined *NPP* as the sum of wood (including coarse roots), foliar, and fine root production. These components were chosen because wood and foliar production comprise 93% of total *NPP* at the Duke FACE site and fine root production is the next largest component (Hamilton *et al.*, 2002). Although our operational definition ignores some components of *NPP* such as leaf volatile emissions and root exudation (Clark *et al.*, 2001), these fluxes are small in these forests (Hamilton *et al.*, 2002; Phillips *et al.*, 2008) and are unlikely to substantially affect estimates of ecosystem *NPP*.

The increment of C in wood, including coarse roots, was calculated by applying allometric regressions from the Duke FACE site (Naidu *et al.*, 1998) to annual measurements of tree diameter, and scaling these values to ground area with the diameter distribution of all trees in the plot as described by Hamilton *et al.* (2002) and DeLucia *et al.* (2005). Regional allometric regressions were used for the hardwood species (Whittaker *et al.*, 1974; Martin *et al.*, 1998; Norby *et al.*, 2001) because site-specific equations were not available. Annual diameter measurements were acquired from increment cores extracted from 20 loblolly pine trees in two dominance classes (suppressed and dominant) in each stand. Two cores were extracted from each tree such that the cores made a 90° angle and the growth increments were averaged. Five individuals of all other tree species that comprised >2% of the total basal area in each stand also were cored using the same procedure.

The cores were sanded, imaged using a flatbed scanner (Epson Perfection V700 Photo, Epson, Rockford, IL, USA) and ring widths were quantified using the Windendro program (Regent Instruments Inc., Nepean, Ontario, Canada). Wood production was calculated for the last 5 years of diameter growth increment and averaged for each tree. To scale hardwood production to the plot level, an allometric equation relating wood production to dbh was developed that included all hardwood species [Supporting Information; Fig. S1;  $\log_{10}(\text{wood production in kg of dry matter}) = -2.0741 + 1.9968 \times \log_{10}(\text{dbh})$ ,  $r^2 = 0.70$ ,  $P < 0.001$ ]. The slope and intercept of this relationship did not vary by species (ANCOVA,  $P > 0.5$ ).

Estimating fine root production represents a significant challenge (Raich & Nadelhoffer, 1989; Majdi *et al.*, 2005; Hendricks *et al.*, 2006), but fine root production in these pine forests is low (Matamala & Schlesinger, 2000; Hamilton *et al.*, 2002), so errors estimating root production have a relatively small effect on *NPP*. We quantified fine root production with a C flux model based on the mean residence time (*MRT*) of C in fine roots (Matamala *et al.*, 2003). Fine root biomass was measured from 10 soil cores (5 cm diameter, 30 cm deep) sampled randomly from each stand in August 2008. Fine roots (<2 mm diameter) were separated from soil, sorted by size, dried, weighed, and analyzed for C content as in Matamala & Schlesinger (2000). The *MRT* for fine roots from Matamala *et al.* (2003) was multiplied by this fine root biomass measurement for each plot to estimate the rate of fine root production. We were not able to visually separate fine roots by species, so we assumed that the proportion of hardwood fine roots to pine fine roots was the same as the proportion of hardwood leaf area index (*LAI*) to pine *LAI* (Vogt *et al.*, 1987; Jackson *et al.*, 1997), and weighted the *MRTs* accordingly. We investigated the magnitude of the uncertainty regarding the choice of root *MRTs* by recalculating fine root production using the pine and sweetgum *MRTs* alone (i.e. assuming 100% pine fine roots or 100% hardwood fine roots).

Foliar production was measured assuming leaf production was equal to litterfall at an annual time-step by collecting litter bi-monthly from ten 0.22 m<sup>2</sup> litter traps per plot as in Finzi *et al.* (2001). Deciduous leaves and needles were separated, dried, ground, and analyzed for C content using an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA, USA). Hardwood leaves were collected from July 14, 2007 to March 17, 2008. Compared with the long-term monthly litterfall collections at the Duke FACE site, these collections captured >90% of the annual total for deciduous leaves. Because loblolly pines hold their needles for ~18 months (Zhang & Allen, 1996), we collected pine needles from July 14, 2007 to June 25, 2009 and scaled the resulting data to an annual increment.

### Stem volume and surface area

The allometric equation used to relate pine diameter to biomass (Naidu *et al.*, 1998) only includes trees up to 30 cm dbh and we were not permitted to harvest larger individuals. To investigate potential bias regarding trees larger than 30 cm dbh, we optically measured (Spiegel Relaskop, Forestry Sup-

pliers, Jackson, MS, USA) the stem volume and surface area of 41 pines that varied from 10 to 110 cm dbh. Briefly, we measured the diameter of the stem at a number of heights up the tree and approximated the surface area and volume of each section as the frustum of a cone. The measured stem volume was nearly identical to the stem volume predicted using the allometric equations for smaller trees (measured volume = 0.9903 × predicted volume + 0.0198,  $r^2 = 0.981$ ,  $P < 0.001$ ; slope not significantly different than 1.0,  $P > 0.8$ ; data not shown). Given that the stem comprises ~90% of above-ground woody biomass in this species (Naidu *et al.*, 1998; Hamilton *et al.*, 2002), we conclude that the allometry of Naidu *et al.* (1998) can be applied to loblolly pine trees larger than 30 cm dbh to accurately predict wood mass.

### LAI

*LAI* (m<sup>2</sup> leaf area m<sup>-2</sup> ground area) was measured at 10 locations within each stand with a canopy analyzer (*LAI* 2000, Li-Cor Biosciences, Lincoln, NE, USA) at approximately monthly intervals. Measurements of *LAI* were corrected for foliar clumping according to Chen (1996) using 10 hemispherical photographs per plot and the HEMIVIEW computer program (version 2.1, Delta-T Devices, Cambridge, UK). *LAI* also was corrected for light interception by stems using the measurements of surface area described previously to calculate the woody area index for each plot; this index was subtracted from the canopy analyzer measurements to estimate *LAI* (Chen, 1996). The relative contribution of pines and hardwoods to total *LAI* was estimated from the litter collections. Hardwood foliar mass was converted to leaf area using measurements of specific leaf area from the Duke Forest (Reich *et al.*, 1999; DeLucia & Thomas, 2000; Pataki & Oren, 2003) after correcting for petiole mass. Pine *LAI* was estimated as the difference between summer maximum ecosystem *LAI* (optical method) and hardwood *LAI* (litterfall method).

### Wood respiration

We measured the respiration of trunk sapwood as an independent estimate of the age-related trajectory of pine  $R_a$  as inferred from the difference of pine *GPP* and *NPP*. We measured CO<sub>2</sub> efflux from eight pine tree trunks at six sites on June 26 and July 10, 2009, at 1.4 m height as in Moore *et al.* (2008) using a closed-path gas exchange system (Li 6400-09, Li-Cor Biosciences). Measurements were performed at night (20:00–02:00 hours) during conditions of minimal sap-flow to minimize measurement bias associated with sap-transport of respired CO<sub>2</sub> (Teskey & McGuire, 2002). While soil CO<sub>2</sub> interferes with the measurement of wood respiration in some species (Teskey & McGuire, 2002; Aubrey & Teskey, 2009), recent research suggests that this artifact is not present in large conifer trees like those measured here (Ubierna *et al.*, 2009). Efflux rates are reported per unit sapwood volume and instantaneous rates were scaled to annual fluxes using the temperature sensitivity measured at the Duke FACE site ( $Q_{10} = 2.4$ ; Hamilton *et al.*, 2002) and measured stem temperatures. We accounted for sapwood respiration of coarse roots

assuming the same tissue-specific rate of respiration as stem sapwood as in Hamilton *et al.* (2002). As branch sapwood often exhibits higher tissue-specific rates of CO<sub>2</sub> production (Cavaleri *et al.*, 2006), we assumed branch sapwood respired at 2.52 times the rate of stem sapwood (Maier *et al.*, 1998) as has been measured previously for this species (Hamilton *et al.*, 2002).

## GPP

To avoid the methodological concerns involved in calculating GPP as the sum of NPP and R<sub>a</sub>, particularly issues related to scaling R<sub>a</sub> to the stand level (Burton & Pregitzer, 2002; Teskey & McGuire, 2002; Davey *et al.*, 2004; Zha *et al.*, 2004; Davidson *et al.*, 2006; Drake *et al.*, 2008), we estimated GPP directly using the canopy conductance approach of Schäfer *et al.* (2003) in five sites that captured the age-related change in NPP. This approach involves calculating a canopy-weighted stomatal conductance from measurements of stem sap-flow (Ewers *et al.*, 2001) for use in the following diffusion equation:  $A_{\text{net}} = G_c \times c_a(1 - c_i/c_a)$ ; where  $A_{\text{net}}$  is the net photosynthesis in  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ,  $G_c$  the canopy-weighted stomatal conductance to CO<sub>2</sub> in  $\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ,  $c_a$  the atmospheric CO<sub>2</sub> concentration ( $\mu\text{mol mol}^{-1}$ ), and  $c_i$  the CO<sub>2</sub> concentration in the air space within leaves. The canopy conductance approach is particularly appropriate in these forests, as photosynthetic rates are strongly related to stomatal conductance, while  $c_i/c_a$  is relatively constant (Katul *et al.*, 2000). Measurements were made between May and September 2008. These months account for 62–66% of annual gross ecosystem productivity of the young pine forest at Duke FACE and 62–67% of this flux in a nearby old hardwood forest as measured by the eddy-covariance (Stoy *et al.*, 2008); thus measurement of canopy photosynthesis during these months captures much of the annual flux even with substantial interannual variation in gross ecosystem production (Stoy *et al.*, 2008). To estimate annual GPP, measured canopy photosynthesis was multiplied by 1.36 assuming that we had measured 64% of annual canopy photosynthesis (Stoy *et al.*, 2008).

## Environmental and structural measurements required for $G_c$

A number of ancillary measurements were required to calculate  $G_c$  from stem sap-flow. 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 vapor pressure deficit ( $D$ ) was calculated from relative humidity and air temperature. Stem temperatures of three pines per plot were measured at 2 and 10 cm depth. Environmental measurements were sampled every 30 s and 1 h averages were recorded with a datalogger (CR10x or CR1000, Campbell Scientific). Incident photosynthetically active radiation (PAR) was measured at the Duke FACE site (Li-190, Li-Cor Biosciences).

Sapwood area was determined from cores extracted with an increment borer from 41 pine trees that encompassed the size distribution of trees from the youngest to the oldest stands;

two cores were taken per tree such that the cores were perpendicular. Sapwood area and heartwood area were differentiated by staining with a 10% ferric chloride solution (Kutscha & Sachs, 1962). An equation was derived to predict sapwood area (cm<sup>2</sup>) from dbh (cm):  $\text{sapwood area} = 18.24 \cdot \text{dbh} - 258.05$ ;  $r^2 = 0.81$ ,  $P < 0.001$ ,  $n = 41$ . This equation was combined with the Relakop measurements (see section 'Stem volume and surface area') to derive an allometric relationship between dbh and total sapwood volume (sapwood volume (m<sup>3</sup>) =  $-0.044 + 1.145 / (1 + \exp(-(dbh - 31.24) / 7.055))$ ),  $r^2 = 0.86$ ,  $P < 0.001$ ,  $n = 41$ ) assuming that the relationship between dbh and sapwood depth did not vary with height (Pruyn *et al.*, 2003).

## Measurement of canopy-weighted stomatal conductance ( $G_c$ )

Sap flux per unit sapwood area ( $J_s$ ,  $\text{g m}^{-2} \text{ s}^{-1}$ ) was measured using the heat dissipation method (Granier, 1987) in five stands of varying age (14, 19, 36, 71, and 97 years of age). We calculated the maximum temperature difference, which operationally defines conditions of zero sap-flow, according to Oishi *et al.* (2008) to allow for nighttime transpiration (Fisher *et al.*, 2007). Eight pines were measured per site and four individuals of the most common hardwood species were measured in the three older stands. *L. tulipifera* and *L. styraciflua* were measured in the 36- and 71-year-old stands, and *L. styraciflua*, *Carpinus caroliniana*, and *A. rubrum* were measured in the 97-year-old stand. The two youngest stands did not have hardwoods of sufficient size to instrument with sap-flow probes; hardwoods contributed <3% of total basal area in these stands. Thus, we assumed that the small individuals in the understory provided a negligible contribution to total canopy photosynthesis.

To estimate radial and circumferential variation in  $J_s$  (Phillips *et al.*, 1996; Ewers & Oren, 2000; Wullschlegel & King, 2000), two trees of each species per plot were instrumented with sap-flow probes on the north and south side at 0–20 mm depth, and two trees of each species per plot were instrumented with probes at 0–20 and 20–40 mm depth on the north side only. There was no difference between  $J_s$  measured on the north and south facing sides (paired  $t$ -test,  $P > 0.1$ ), so we did not apply circumferential corrections.  $J_s$  was lower in the 20–40 mm depth than the 0–20 mm depth (26% for pines, 16% for hardwoods, paired  $t$ -test,  $P < 0.05$ ), so we applied depth corrections as in Phillips *et al.* (1996) and Schäfer *et al.* (2002). There was no evidence of seasonal or diurnal variation in the magnitude of this difference. Thus, we estimated  $J_s$  for sapwood deeper than 20 mm by dividing the measurement of  $J_s$  at 0–20 mm depth by 1.26 (pines) or 1.16 (hardwoods). This was carried out individually for each tree to capture tree-level variation in sapwood depth.

Total sap flux in each plot ( $E_t$ ) was calculated by applying the mean hourly measurements of  $J_s$  of each species to each individual in the plot, thus accounting for the distribution of sapwood depth in noninstrumented trees. Canopy transpiration ( $E_c$ ;  $\text{mmol m}^{-2} \text{ s}^{-1}$ ) was calculated as  $E_t$  divided by plot area. The mean canopy stomatal conductance

( $G_c$ ;  $\text{mmol m}^{-2} \text{s}^{-1}$ ) was calculated as follows (Ewers *et al.*, 2001):  $G_c = K_G(T) \cdot E_c / (D \cdot \text{LAI}_{\text{pine}})$ ; where  $D$  is the vapor pressure deficit and  $K_G$  the conductance coefficient that accounts for the temperature sensitivity of the psychrometric constant, latent heat of vaporization, air density, and air specific heat ( $115.8 + 0.4236T$ ,  $\text{kPa m}^3 \text{kg}^{-1}$ ). To keep measurements errors below 10%, values for  $G_c$  were calculated only when  $D \geq 0.6 \text{ kPa}$  (Ewers & Oren, 2000).

### $c_i/c_a$

We used the stable C isotopic composition of wood as a measure of temporally integrated  $c_i/c_a$  (Farquhar *et al.*, 1982; Katul *et al.*, 2000; Dawson *et al.*, 2002). The last 5 years of wood from the cores used to measure wood production was separated, dried, ground, and analyzed for  $\delta^{13}\text{C}$  at the University of Illinois using an elemental analyzer (ECS 4010, Costech Analytical) coupled to a ConFlo IV interface (Thermo, Bremen, Germany) and a Delta-V advantage isotope-ratio mass spectrometer (Thermo). We analyzed eight pines at all 12 sites and 10 of each hardwood species for the five sites where sap-flow was measured. The precision calculated from 12 samples run in duplicate was within 0.09‰ and the average measured deviation of an in-house isotopic reference material was <0.05‰ of its known value ( $n = 5$ ).

We estimated  $c_i/c_a$  from wood  $\delta^{13}\text{C}$  using the simple model of Farquhar *et al.* (1982) assuming atmospheric  $\delta^{13}\text{C}$  was  $-8\text{‰}$  (Battle *et al.*, 2000). We follow Katul *et al.* (2000) and Schäfer *et al.* (2003) and assume that this  $c_i/c_a$  value reflects the long-term light-saturated  $c_i/c_a$ . For light levels below  $700 \mu\text{mol m}^{-2} \text{s}^{-1}$ , we estimated  $c_i/c_a$  using the hybrid model of Katul *et al.* (2000). We used wood  $\delta^{13}\text{C}$  derived estimates of  $c_i/c_a$  because these values integrate over long time periods, which is appropriate for our focus on annual fluxes. However, wood  $\delta^{13}\text{C}$  can vary because of factors other than  $c_i/c_a$  (e.g. wood chemical composition; van Bergen & Poole, 2002), so we compared wood  $\delta^{13}\text{C}$  derived  $c_i/c_a$  with direct measurements from foliar gas exchange as an independent check on the  $c_i/c_a$  estimates (see section 'Evaluations of assumptions used to calculate  $G_c$  and GPP', Drake *et al.*, 2010).

### Evaluation of assumptions used to calculate $G_c$ and GPP

Many assumptions are required to estimate  $G_c$  from sap-flow measurements and to estimate GPP from  $G_c$  and  $c_i/c_a$ . These assumptions include: (1) air temperature approximates leaf temperature. This was tested with direct measurements of leaf temperature and comparison against aerodynamic conductance in these pine forests. This assumption may not hold for understory hardwood trees. (2) We assumed that water storage in the stem between the measurement location of  $J_s$  and the transpiring leaves did not decouple measurements of  $J_s$  from canopy C uptake. If this occurred, we would observe a time-lag between increasing VPD and  $J_s$  that would lengthen with tree size and age. However, we were unable to detect such a lag at the hourly timescale of our  $J_s$  measurements. (3) We assumed that wood  $\delta^{13}\text{C}$  provides a reliable time-integrated estimate of

canopy-average  $c_i/c_a$ . Hu *et al.* (2010) used a similar approach to estimate GPP in a conifer-dominated forest and made extensive measurements of branch carbohydrate  $\delta^{13}\text{C}$  to estimate canopy  $c_i/c_a$ . They found that the final GPP estimates were more dependent on  $G_c$  and not  $c_i/c_a$ , which was relatively constant. A synthesis of extensive gas-exchange measurements of loblolly pine (Katul *et al.*, 2000) demonstrated remarkably constant light-saturated  $c_i/c_a$  values centering on 0.66, which is in agreement with our estimate derived from wood  $\delta^{13}\text{C}$  (see 'Results'). (4) We assumed a fixed  $\text{CO}_2$  concentration of  $385 \mu\text{mol mol}^{-1}$ , as turbulent mixing within the rough canopy of these forests produces a stable profile of  $\text{CO}_2$  concentration during daylight hours (Katul & Albertson, 1999).

As a final check of the validity of these assumptions, we investigated the ability of the sap-flow approach to reliably predict  $G_c$  by measuring light-saturated stomatal conductance of top canopy pine needles from July 12 to July 23, 2008 in the five stands where sap-flow was measured simultaneously. As these sites lacked canopy access, we sampled canopy foliage with a shotgun. To ensure that detached needles maintained physiological activity, we measured photosynthesis on attached canopy needles using access towers at the Duke FACE site and determined that stomatal conductance was stable after cutting for >45 min when branches were sampled before 13:00 hours (Drake *et al.*, 2010). Detailed descriptions of these methods and data are available (Drake *et al.*, 2010). We were unable to obtain reliable gas exchange measurements on detached hardwood leaves and thus only report pine gas exchange data. Eight to 10 upper canopy branches were shot down in the midmorning through the early afternoon (10:00–13:00 hours); stomatal conductance was measured <10 min after harvesting with open gas exchange systems (Li 6400, Li-Cor Biosciences) under saturating irradiance ( $1500 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$ , 6400-02B red/blue light source,  $2 \times 3 \text{ cm}$  chamber, Li-Cor Biosciences) and ambient  $\text{CO}_2$  ( $385 \mu\text{mol mol}^{-1}$ ). The needle area inside the chamber was isolated and photographed for the measurement of projected leaf area using the IMAGEJ program (Abramoff *et al.*, 2004). The calculation of  $G_c$  implicitly assumes that leaf temperature was equal to air temperature to estimate the diffusional gradient of  $\text{H}_2\text{O}$  from measurements of vapor pressure deficit in the canopy air-space. This assumption was corroborated by measurements of leaf temperature during these gas exchange measurements; leaf temperature was strongly correlated with air temperature with a slope not significantly different than 1.0 and an intercept not significantly different than zero (leaf temperature =  $1.07 \times \text{air temperature} - 1.18$ ,  $r^2 = 0.87$ ,  $P < 0.001$ ,  $n = 71$ ).

The comparison of leaf-level  $g_s$  to canopy-level  $G_c$  implicitly assumes that boundary-layer conductance is much larger than  $g_s$ , as  $G_c$  includes boundary-layer conductance. We assume that canopy conductance is approximately equal to canopy-weighted stomatal conductance ( $G_c$ ). Canopy conductance is expressed as  $G_c = (g_s \times g_a) / (g_s + g_a)$ , where  $g_a$  is aerodynamic conductance. This expression can be rewritten as  $G_c = (g_s \times g_a) / [g_a(1 + g_s/g_a)] = g_s / (1 + g_s/g_a)$ ; so as long as  $g_s/g_a \ll 1$ , then  $G_c$  is approximately equal to  $g_s$ . Estimates of  $g_a$  for pine canopies from LAI of 3 to 6 vary from 3.5 to  $1.1 \text{ mol m}^{-2} \text{s}^{-1}$  (G. Katul, personal communication, Kelliher

*et al.*, 1993; Juang *et al.*, 2007). Even the lower values of  $g_a$  are more than an order of magnitude greater than  $g_s$  for loblolly pine (Ellsworth *et al.*, 1995; Drake *et al.*, 2010). Thus, it is reasonable to assume that loblolly pine canopies are closely coupled with the atmosphere ( $g_s \ll g_a$ ) and  $G_c$  primarily reflects  $g_s$ .

### Data analysis

Many previous investigations have treated forest age as a categorical variable with replicate plots within age-classes and used analysis of variance (ANOVA) for statistical inference (e.g. Pregitzer & Euskirchen, 2004). We chose to follow the continuous nature of forest age and used a regression framework for statistical inference; the benefit of this approach is that the timing of age-related changes in ecosystem processes can be compared to aid interpretation. Stands were treated as the unit of replication in all cases, with each stand adding 1 df to the regression 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 for all analyses using the UNIVARIATE and REG procedures. We calculated the uncertainty of our pine wood production estimates with a Monte Carlo re-sampling technique, where the distribution of wood production by individual pine trees was defined as a Poisson distribution derived from the observed variance at each site. We report the average standard deviation of 20 samples randomly obtained from this distribution, performed 100 times as outlined in Harmon *et al.* (2007). We calculated the uncertainty in our estimate of wood production by hardwoods by propagating the uncertainty in the production allometry (Fig. S1, see above, 'Production measurements'). One thousand samples of the slope and intercept were drawn from a normal distribution defined by the mean and standard deviation of the observed regression; these parameters were then applied to every hardwood; we present the standard deviation of these 1000 estimates. The standard deviation of fine-root and foliar production was scaled from subreplicates within each plot. The standard deviation of aggregate production terms (e.g. *NPP*) was estimated by error propagation as in Harmon *et al.* (2007).

## Results

### Stand characteristics

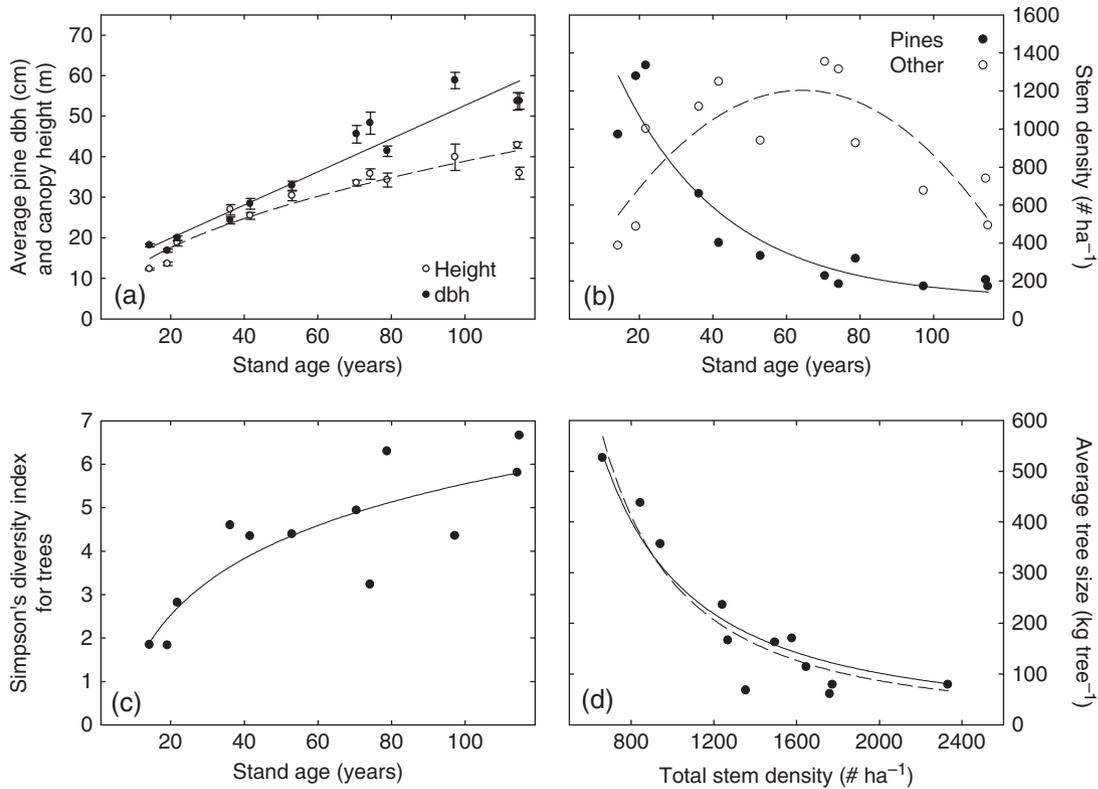
Site selection minimized the variation in stand structural attributes that were not age related. The average pine dbh increased linearly with age (Fig. 1a;  $y = 12.94 + 0.42x$ ,  $r^2 = 0.91$ ,  $P < 0.001$ ), while canopy height increased as a power function (Fig. 1a;  $y = 3.65x^{0.52}$ ,  $r^2 = 0.94$ ,  $P < 0.001$ ); there was little non-age-related variation in these structural characteristics. Loblolly pine was by far the most numerous species in the youngest stands, but their density decreased early in stand development [Fig. 1b;  $y = 106 + 1925 \times \exp$

$(-0.0346x)$ ,  $r^2 = 0.83$ ,  $P < 0.001$ ]. At the same time, the number of small understory hardwood individuals increased in abundance, notably *L. styraciflua*, *A. rubrum*, *L. tulipifera*, *Quercus alba*, and *Carya* spp. (Fig. 1b, 20–40 years;  $y = -0.27x^2 + 34.67x + 91.55$ ,  $r^2 = 0.71$ ,  $P < 0.01$ ). Later in development, from 80 to 90 years of age, the abundance of hardwoods decreased, presumably because of competition for canopy access. The oldest sites had a mixed canopy of a few, large pines and many medium to large hardwoods trees. This change in community composition was reflected in the Simpson's diversity index, which increased from 2 in the youngest forests to 6 in the  $\sim 115$ -year-old stands (Fig. 1c;  $y = -4597 + 4594x^{0.0004}$ ,  $r^2 = 0.65$ ,  $P < 0.01$ ). The stands fell along the '-3/2 curve' (dashed curve, Fig. 1d;  $y = 3.6 \times 10^7 x^{-1.7}$ ,  $r^2 = 0.9$ ,  $P < 0.01$ ; Weller, 1987; Zeide, 1987), indicating that these stands were undergoing the natural process of self-thinning.

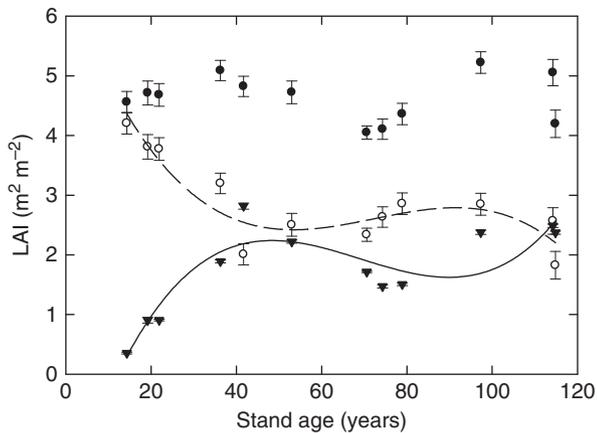
Maximum summer *LAI* was 4–5 and was nearly constant with age (Fig. 2). The components of *LAI*; however, changed markedly. Hardwood *LAI* increased from 15 to 40 years, declined from 40 to 80 years, and then increased up to 115 years, mirroring the changes in stem density (Fig. 2;  $y = -2.3 + 0.23x - 0.0036x^2 + 1.7 \times 10^{-5}x^3$ ,  $r^2 = 0.73$ ,  $P < 0.01$ ). Pine *LAI* followed the opposite pattern (Fig. 2;  $y = 6.66 - 0.19x + 0.0029x^2 - 1.3 \times 10^{-5}x^3$ ,  $r^2 = 0.78$ ,  $P < 0.01$ ). Pine *LAI* comprised nearly 100% of the total in the youngest forests, but the canopy in stands of 40–115 years of age was comprised of roughly equal amounts of pine and hardwood foliage. *LAI* and other data describing stand characteristics are available (Table S1).

### NPP

Total *NPP* declined strongly with increasing age early in development (Fig. 3a), from  $961 \text{ g C m}^{-2} \text{ yr}^{-1}$  in the youngest forest to  $\sim 600 \text{ g C m}^{-2} \text{ yr}^{-1}$  in forests from 50 to 115 years of age [ $y = 491.3 + 2722.9 \times \exp(-0.12x)$ ,  $r^2 = 0.9$ ,  $P < 0.001$ ]. This decline was exclusively driven by a decline in pine production [ $y = 252.1 + 2590.5 \times \exp(-0.094x)$ ,  $r^2 = 0.94$ ,  $P < 0.001$ ], and partially alleviated by increasing hardwood production (Fig. 3a;  $y = -13.04 + 173.37 / (1 + \exp(-(x-24.42)/6.21))$ ,  $r^2 = 0.88$ ,  $P < 0.001$ ). The decline in pine *NPP* was largely driven by an 83% decline in pine wood production [Fig. 3b;  $y = 114 + 2529.1 \times \exp(-0.098x)$ ,  $r^2 = 0.95$ ,  $P < 0.001$ ], but a small decline in pine needle production (Fig. 3c) also was observed ( $y = 177.5 - 0.45x$ ,  $r^2 = 0.42$ ,  $P < 0.05$ ). Hardwood wood (Fig. 3b;  $-1.96 + 80.18 / (1 + \exp(-(x-30.84)/7.31))$ ,  $r^2 = 0.91$ ,  $P < 0.001$ ) and foliar production (Fig. 3c;  $y = -101 + 9.48x - 0.15x^2 + 9.48x^3$ ,  $r^2 = 0.86$ ,  $P < 0.001$ ) increased in equal proportions. Fine root production was low in all of the sites but increased



**Fig. 1** Characteristics of 12 forest stands that vary in age in East-central North Carolina. Average canopy height and diameter at breast height of dominant loblolly pine (*Pinus taeda*) trees (a); error bars reflect  $\pm 1$  SE. Stem density of pines (solid circles) and other trees dominated by mid- and late-successional hardwood species (open circles) (b) and Simpson's diversity index (c) with increasing age. The relationship between average tree size and stem density (d) shows that all stands fall near the '-3/2 thinning curve' reflected by the solid curve; the dashed curve is the best fit to the data.

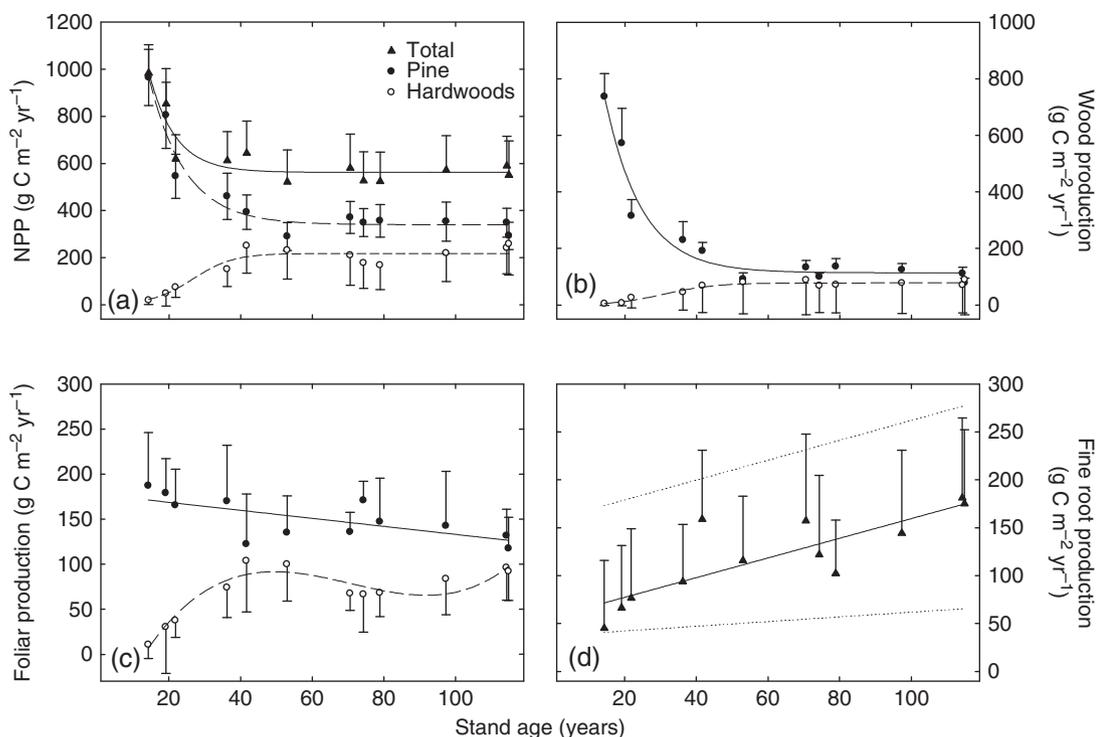


**Fig. 2** Leaf area index (LAI) of 12 forest stands that vary in age corrected for woody interception and foliar clumping (see Methods). Ecosystem LAI was measured optically, hardwood LAI was estimated from litter collections, and pine LAI was estimated by difference. Error bars reflect  $\pm 1$  SE of 10 measurements per plot.

with age (Fig. 3d;  $y = 29 + 0.52x$ ,  $r^2 = 0.66$ ,  $P < 0.001$ ). The proportion of NPP contributed by fine roots increased with age from 2% of total NPP in the youngest forest to 19% of total NPP in the oldest forest.

*Canopy conductance, photosynthesis, GPP, and R<sub>d</sub>*

Canopy-weighted stomatal conductance for pines and hardwoods corresponded with precipitation throughout the measurement period (Fig. 4a and b). Daily average pine canopy conductance generally declined with age, while hardwood conductance increased. Averaged across the entire measurement period, daytime pine conductance was 0.037, 0.050, 0.039, 0.027, and 0.020 mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> in the stands of 14, 19, 36, 71, and 97 years of age, respectively; hardwood conductance was 0.024, 0.061, and 0.074 mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> in the stands of 36, 71, and 97 years of age, respectively. Canopy-weighted conductance for pine was highly correlated with conductance measured by gas exchange (Fig. 4c, gas exchange  $g_s = 1.18 \times \text{sap-flow } G_s + 0.01$ ,



**Fig. 3** Net primary production (*NPP*) and its components in 12 forest stands that vary in age. Total *NPP* (a) was the sum of wood (b), foliar (c), and fine root (d) production. The data in (d) were calculated assuming that the ratio of pine to hardwood fine root biomass followed the ratio of *LAI*; dotted lines represent the maximum uncertainty related to the timescale of fine root turnover: the lower dashed line represents the estimate of fine root production assuming all roots were pine and thus had a long mean residence time, whereas the upper dashed line represents the estimate assuming all roots belong to *Liquidambar styraciflua* and thus had a short mean residence time. Error bars reflect  $\pm 1$  SD and were estimated from subreplicate measurements (c–d), Monte-Carlo simulation (b; see Methods), or error propagation (a).

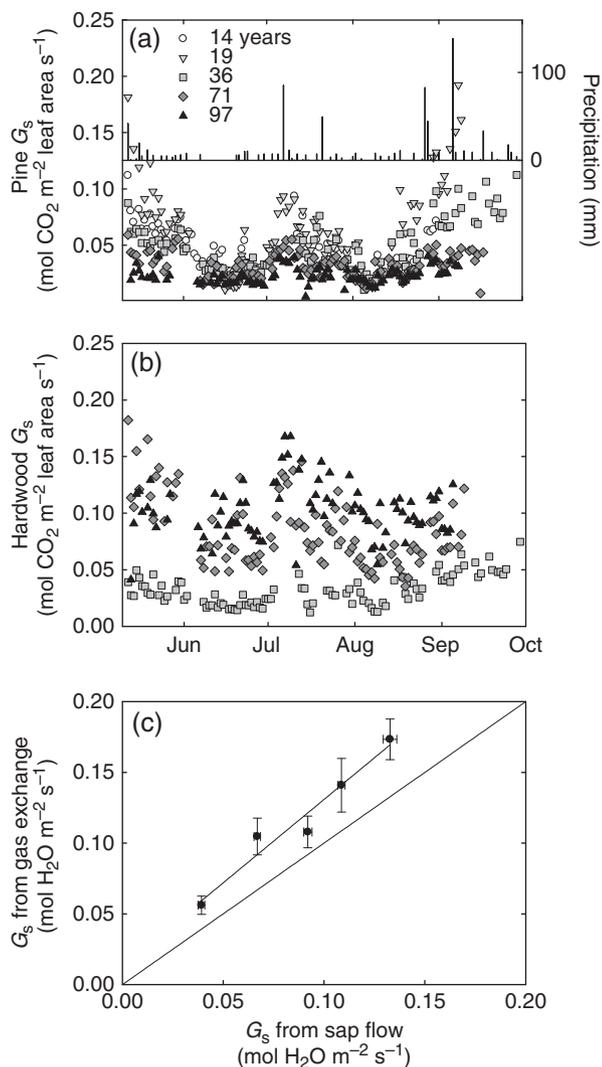
$r^2 = 0.94$ ,  $P < 0.001$ ). Conductance from gas exchange was significantly higher than the sap-flow measurements, but this was expected; we measured gas exchange on top canopy sun leaves that typically have the highest levels of conductance, while the sap-flow method integrates over the entire canopy and includes shade foliage with lower conductance (Harley *et al.*, 1996; McDowell *et al.*, 2002; Ambrose *et al.*, 2009; Mullin *et al.*, 2009). Additionally, our estimate of average  $G_c$  in the 19-year-old stand was similar to a separate study of sap-flow derived  $G_c$  at the nearby Duke FACE site when that forest was 20-years-old (Schäfer *et al.*, 2003). We conclude that the sap-flow method produced reliable estimates of canopy-weighted stomatal conductance.

Pine wood  $\delta^{13}\text{C}$  increased with age from  $-28\text{‰}$  at 15 years of age to  $-26.5\text{‰}$  at 115 years of age (Fig. 5a;  $y = -27.29 + 0.0125x$ ,  $r^2 = 0.45$ ,  $P < 0.05$ ). This corresponds to a decline in  $c_i/c_a$  with age, from 0.65 at age 15 to 0.58 at age 115 (Fig. 5b;  $y = 0.65 - 0.0005x$ ,  $r^2 = 0.45$ ,  $P < 0.05$ ). Hardwood  $\delta^{13}\text{C}$  did not vary with age or canopy position (data not shown); the average isotopic composition was  $-31.89\text{‰}$ , corresponding to a  $c_i/c_a$  of

0.82. These estimates of pine  $c_i/c_a$  were significantly correlated with independent measurements of  $c_i/c_a$  from leaf-level gas exchange [isotopic  $c_i/c_a = 0.321 \times (\text{gas exchange } c_i/c_a) + 0.442$ ,  $r^2 = 0.39$ ,  $P < 0.05$ ; Drake *et al.*, 2010], suggesting that wood  $\delta^{13}\text{C}$  reflects leaf  $c_i/c_a$ .

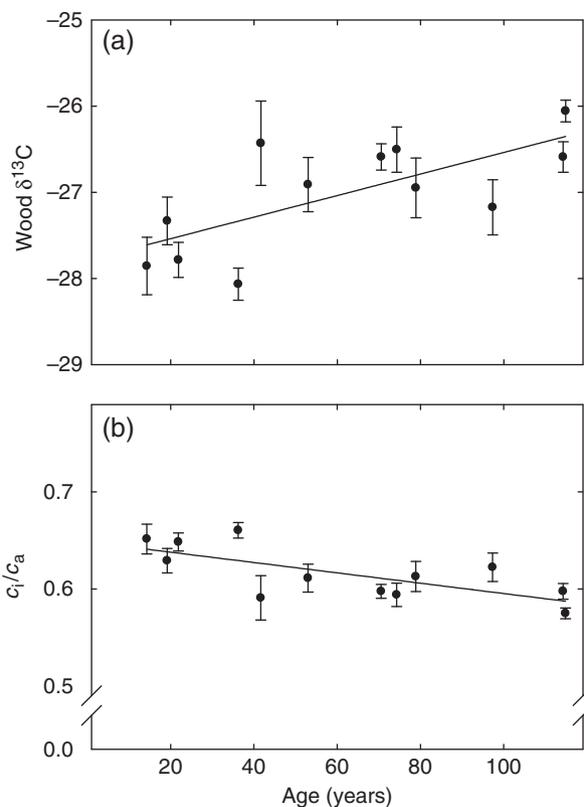
Canopy photosynthesis mirrored the age-related change in conductance (Fig. 6a); pine photosynthesis declined after 19 years ( $y = 1472.5 - 10.3x$ ,  $r^2 = 0.93$ ,  $P < 0.01$ ), while hardwood photosynthesis increased ( $y = 11.8x - 413.6$ ,  $r^2 = 0.96$ ,  $P = 0.08$ ). The decline in pine photosynthesis decreased total stand photosynthesis through 71 years of age, but increasing hardwood photosynthesis in the 97-year-old stand caused stand photosynthesis to be equivalent in the youngest and oldest forests.

As the decline in ecosystem *NPP* was driven by the pines, we focused on the pine C fluxes. *GPP*, *NPP*, and  $R_a$  decreased with age when the measured pine canopy photosynthesis was scaled to an annual flux (Fig. 6b; pine  $GPP = 2337 - 16.3 \times \text{age}$ ,  $r^2 = 0.93$ ,  $P < 0.01$ ). The decline in  $R_a$  (estimated from the difference between *GPP* and *NPP*) was corroborated by a decline in annual



**Fig. 4** Canopy-weighted stomatal conductance for CO<sub>2</sub> ( $G_s$ ) of loblolly pine (a) and hardwood trees (b) in forest stands of varying age derived from sap-flow measurements. Symbols refer to daily daytime averages. Canopy-weighted stomatal conductance for H<sub>2</sub>O ( $G_w$ ) was compared with stomatal conductance measured on top canopy pine needles ( $g_s$ ) in July (c). Error bars reflect  $\pm 1$  SE, and the 1:1 line is shown for reference.

sapwood respiration. The tissue-specific rate of stem sapwood respiration declined with age (Fig. 7a;  $y = 51.37 - 0.256x$ ,  $r^2 = 0.86$ ,  $P < 0.01$ ) as is frequently reported (Yoda, 1967; Carey *et al.*, 1997; Prunyn *et al.*, 2002a,b) and measured rates in young forests corresponded well with measurements from the Duke FACE site (Hamilton *et al.*, 2002). This decline in the tissue-specific respiration rate overwhelmed the age-related increase in sapwood volume. Stand-level respiration of stem, branch, and coarse root sapwood declined after 50 years of age (Fig. 7b;  $y = 267 + 2.34x - 0.017x^2$ ,  $r^2 = 0.27$ ,  $P = 0.09$ ), which corresponds well with the timing of the

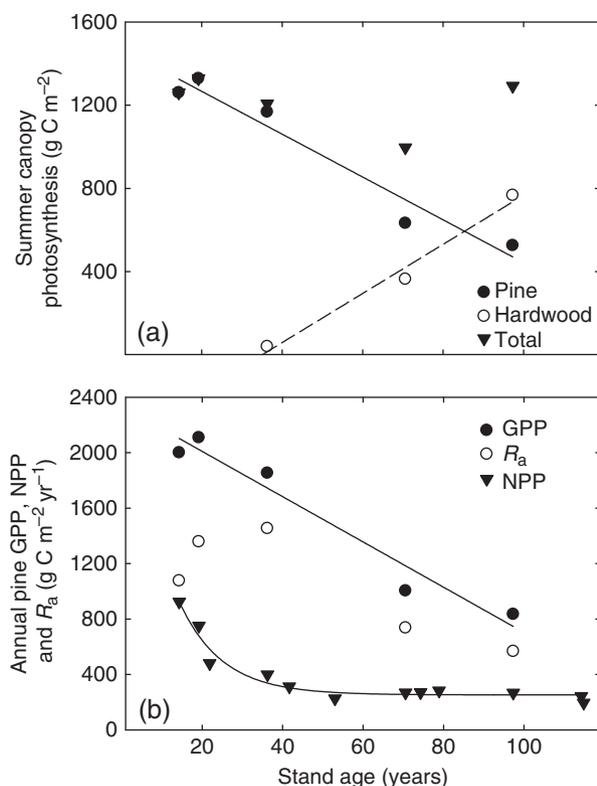


**Fig. 5** Pine wood carbon isotopic composition and corresponding time-integrated  $c_i/c_a$  values;  $c_i$  refers to the CO<sub>2</sub> concentration inside needle airspaces, whereas  $c_a$  refers to atmospheric CO<sub>2</sub> concentration. Wood  $\delta^{13}C$  (a) was used to calculate  $c_i/c_a$  (b) according to the simple model of Farquhar *et al.* (1982). Error bars reflect  $\pm 1$  SE.

decline in  $R_a$  as calculated by the difference between  $GPP$  and  $NPP$  (Fig. 6b).

## Discussion

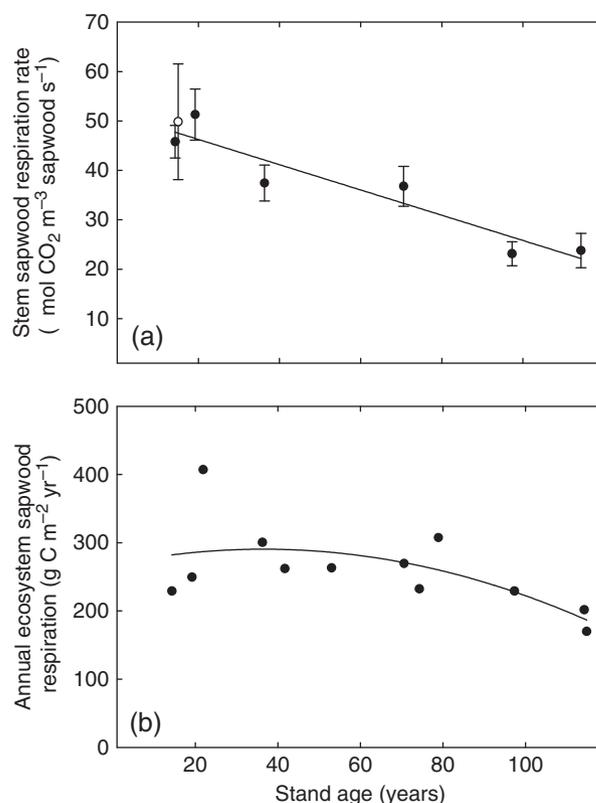
Two major mechanisms affected C cycling of aging loblolly pine forests. At the physiological level, the decline in pine  $GPP$  contributed to lower  $NPP$  in old forest stands, while at the community level, increasing production by hardwood trees partially alleviated the decline in pine production. In contrast to the 'respiration hypothesis' (Möller *et al.*, 1954; Yoda *et al.*, 1965; Kira & Shidea, 1967; Whittaker & Woodwell, 1967; Odum, 1969), declining  $GPP$  rather than increasing  $R_a$  caused  $NPP$  to decline as pines aged. Sapwood respiration, a major component of  $R_{av}$ , also declined with age. The decrease in pine  $GPP$  was driven by the age-related decrease in canopy conductance, supporting the hydraulic limitation hypothesis of Ryan *et al.* (2006). The age-related decline in pine  $NPP$  was partially offset by



**Fig. 6** Summer canopy photosynthesis and annual pine C fluxes for forest stands of varying age. Canopy photosynthesis during the measurement period (a) was scaled to annual estimates of gross primary production (GPP) by loblolly pines (b). The net primary production (NPP) values are re-plotted from Fig. 3a. Autotrophic respiration ( $R_a$ ) was calculated as the difference between GPP and NPP.

the increase in production of hardwoods, highlighting the importance of successional changes in community composition on forest C cycling.

The chronosequence approach employed in this research involves two fundamental assumptions: (1) site selection minimized non-age-related differences between plots and (2) all plots would recreate the trajectory of change inferred from the chronosequence if measurements were repeated over time. Recently, Johnson & Miyanishi (2008) questioned the validity of these assumptions in several classic studies of succession. Regarding the first assumption, the high coefficients of determination ( $>0.9$ ) reported for variables (e.g. canopy height, dbh, production terms) regressed against age suggest that our selection of sites indeed minimized non-age-related differences between plots. The second assumption is inherently more difficult to address. However, the assumption was supported by substantial overlap among sites in tree core-derived timelines of pine wood production, suggesting a common trajectory of wood production across all sites:



**Fig. 7** Stem sapwood respiration in loblolly pine forest stands of varying age. The instantaneous tissue-specific rate of respiration at  $25^\circ\text{C}$  (a) by pines in the chronosequence are shown as filled circles; measurements from the Duke Free Air  $\text{CO}_2$  Enrichment site are shown as the open symbol for reference (Hamilton *et al.*, 2002). Error bars reflect  $\pm 1$  SE. The tissue-specific respiration rates were scaled to the annual flux of stem, branch, and coarse root sapwood (b); the regression in (a) was interpolated to predict the tissue-specific rate at sites where it was not measured directly.

wood production increased linearly up to a peak at  $\sim 15$  years, followed by a strong negative exponential decline up to  $\sim 50$  years (Fig. S2). While we recognize that chance events regarding dispersal and disturbance may alter the trajectory of succession in these forests (e.g. the species composition of colonizing hardwood trees; Turner *et al.*, 1998; Clark *et al.*, 2004; Dietze & Clark, 2008), we conclude that these sites offer a valid space-for-time substitution regarding the age-related change in production.

The canopy conductance approach provided reasonable estimates of GPP and may provide a powerful method of obtaining independent estimates of canopy photosynthesis. The value of GPP in the 19-year-old stand estimated by the canopy conductance approach ( $2109 \text{ g C m}^{-2} \text{yr}^{-1}$ ) was similar to values for the 20-year-old ambient  $\text{CO}_2$  plots in the Duke FACE forest made by the summation of NPP and  $R_a$  ( $2371 \text{ g C m}^{-2} \text{yr}^{-1}$ ;

Hamilton *et al.*, 2002) and using a sap-flow-derived model similar to the method used here ( $2139 \text{ g C m}^{-2} \text{ yr}^{-1}$ ; average of 1999 and 2000 for pines; Schäfer *et al.*, 2003). The correspondence between our estimate and these independent estimates of *GPP* suggests that the simplifying assumptions we used were adequately met at an annual time-step. We assumed that variation in photosynthesis through the canopy concerning  $c_i/c_a$  would be captured in the  $\delta^{13}\text{C}$  values of wood. This assumption was supported by a sensitivity analysis (Fig. S3) indicating that the inference of an age-related decline in pine *GPP* was insensitive to variation in assumed  $c_i/c_a$  values. While the absolute magnitude of the *GPP* estimates was dependent on estimates of  $c_i/c_a$ , the decline in  $G_c$  with age was so large that *GPP* always declined, regardless of the  $c_i/c_a$  values.

The age-related decline in pine *GPP* appears to be caused by increasing hydraulic limitation. Drake *et al.* (2010) tested predictions derived from the hydraulic limitation hypothesis (Ryan *et al.*, 2006) in the same forests studied here, and nearly all of the results supported the hypothesis. Leaf-level light saturated photosynthesis declined, leaf- and canopy-level stomatal conductance declined, the concentration of  $\text{CO}_2$  inside leaf airspaces declined, stomatal limitation inferred from  $\text{CO}_2$ -response ( $A:c_i$ ) curves increased, specific leaf area decreased, and the ratio of sapwood area to leaf area increased (Drake *et al.*, 2010). The sensitivity of radial growth to interannual variation in temperature and drought decreased with age, the opposite of predicted, but this was consistent with sap-flow results demonstrating less sensitivity of stomatal conductance to increasing vapor pressure deficit with increasing age. Taken together, these results suggest hydraulic limitation of photosynthesis increased as the *P. taeda* trees aged and grew tall, which reduced pine *GPP* and *NPP*. This result differs from another study linking measurements of whole canopy conductance in forests where *NPP* was known to decline with age; Barnard & Ryan (2003) suggest that reduced photosynthesis from triose phosphate utilization limitation, not increasing hydraulic limitation, reduced *GPP* and *NPP* in an aging *Eucalyptus* forest.

The demonstration that pine *NPP* declined because of declining *GPP* and not increasing  $R_a$  is relevant to a recent literature review. DeLucia *et al.* (2007) compiled all estimates of *NPP* and *GPP* for forests of known age, and demonstrated a decline in *NPP/GPP* with age, suggesting that *NPP* declines in aging forests because of increasing  $R_a$ . However, DeLucia *et al.* (2007) recognized that forest type was confounded with age, as most of the young forests were temperate and tropical plantations, while many of the old forests were from the boreal region. If the loblolly pine results presented here are broadly applicable to global forests, it would sug-

gest that variation in *NPP/GPP* reported by DeLucia *et al.* (2007) was driven by biome differences, and not age *per se*.

At the ecosystem scale, it appears that increasing photosynthesis by later-successional hardwoods may have sustained relatively high rates of *GPP* in the old forest stands. However, the use of the sap-flow method to estimate hardwood *GPP* is potentially problematic, as some of the assumptions that work for pines may not be appropriate for broadleaved trees. In particular, we assumed that leaf temperature was equal to air temperature; this is generally true for thin-needled conifers that are highly coupled with the atmosphere (Katul *et al.*, 1997) and was supported by our measurements, but the increased boundary layer and thermal absorbing surface of broadleaves often leads to higher leaf temperatures relative to air temperature (Leuzinger & Korner, 2007; Helliker & Richter, 2008). If actual hardwood leaf temperatures were systematically higher than measured air temperature we would have overestimated photosynthesis. Thus, while the age-related increase of hardwood photosynthesis is likely robust as it follows the increase in hardwood *LAI* (Fig. 2), the absolute magnitude of these estimates should be interpreted with caution.

The principal role of photosynthesis as a driver of age-related changes in the C cycles of these forests suggests that the increasing concentration of atmospheric  $\text{CO}_2$  may have a more prominent role in controlling the C-sink strength of these forests than increasing temperature. This is opposite of the suggestion of Valentini *et al.* (2000), who found that ecosystem respiration was the main determinant of annual C uptake across a network of forest eddy-covariance sites in Europe. However, a reanalysis of the same forests suggested that respiration was largely a function of productivity, and thus net annual C storage was largely a function of gross C uptake (Janssens *et al.*, 2001). In the future, a physiological ecosystem model incorporating the mechanisms of hydraulic limitation and secondary succession could be used to examine the trajectory of C storage in successional forests under different scenarios of global change.

The suggestion that elevated  $\text{CO}_2$  may increase forest C sequestration by increasing *GPP* also conflicts with the current generation of coupled global circulation models (Friedlingstein *et al.*, 2006; Denman *et al.*, 2007). These models predict a large transfer of C from ecosystems to the atmosphere by temperature-induced increases in heterotrophic and autotrophic respiration that outweigh the  $\text{CO}_2$  fertilization of *GPP*, while the results presented here would suggest the opposite should be true. We suggest that this apparent disparity exists because the current work did not address the

eventual fate of the extra C predicted to enter these forests from CO<sub>2</sub> fertilization. All of the models in Denman *et al.* (2007) exhibit an increasing soil C turnover rate even in the absence of climate change, as the size of decomposing pools increase with no change in their turnover rates. A similar phenomenon of faster C cycling through ecosystems exposed to elevated CO<sub>2</sub> has also been observed (Jackson *et al.*, 2009; Lukac *et al.*, 2009). Additionally, the rapid connection between canopy photosynthesis and rhizosphere respiration (Hogberg *et al.*, 2001; Drake *et al.*, 2008; Savage *et al.*, 2009) suggests that a portion of the extra C fixed under elevated CO<sub>2</sub> may be rapidly returned to the atmosphere. Thus, while the results of this study suggest that elevated CO<sub>2</sub> would stimulate *GPP* and *NPP* of aging successional warm-temperate forests, the implications for long-term C storage are still uncertain, as a stimulation of *GPP* and *NPP* may simply increase the rate at which C cycles through the ecosystem.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Characteristics of twelve forests dominated by loblolly pine (*Pinus taeda*) in East-central North Carolina that differ in age. BA is basal area, Pine refers to *Pinus taeda*, HW refers to a collection of mid- and late-successional hardwood species, dbh is diameter-at-breast-height (1.4 m), and LAI is leaf area index. Max LAI refers to maximum values observed in the summer, typically in August, and min LAI refers to the minimum values observed in the winter, typically in January. **Figure S1.** Allometric relationship used to predict wood production by hardwood trees as a function of diameter at breast height. There was no difference in the slope or intercept of this relationship between species (ANCOVA,  $P > 0.05$ ). The legend gives the genus or four letter genus-species abbreviation. Acru refers to *Acer rubrum* (Red Maple); Cayra refers to all hickory species in the genus; List refers to *Liquidambar styraciflua* (Sweetgum); Litu refers to *Liriodendron tulipifera* (Tulip poplar); and Qual refers to *Quercus alba* (White oak). The line is the best fit to all species:  $y = 1.997x - 2.074$ ;  $r^2 = 0.70$ ;  $P < 0.01$ .

**Figure S2.** Consistent trajectory of pine wood production across chronosequence plots. Average stem diameter increments of 20 dominant and 20 suppressed *Pinus taeda* trees over the last 30 years derived from tree cores as in the Methods were scaled allometrically to wood production and multiplied by the observed stem density. Each plot is shown as a unique symbol. The wood production values used in the main text are shown as large grey circles and represent the average of the last 5 years of woody increment.

**Figure S3.** Sensitivity of *GPP* estimates to light-saturated  $c_i/c_a$ . The estimate derived from wood  $\delta^{13}\text{C}$  was compared to constant values of 0.55, 0.66, and 0.75, as 0.66 reflects the overall mean, while 0.55 and 0.75 reflect the range of *Pinus taeda* leaf-level gas-exchange observations (Katul *et al.*, 2000). While the absolute magnitude of the *GPP* estimate depended on  $c_i/c_a$ , the inference of an age-related decline in *GPP* was insensitive to  $c_i/c_a$  and was driven by a large decline in canopy conductance with increasing age.

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