Rising concentrations of atmospheric CO$_2$ have increased growth in natural stands of quaking aspen *(Populus tremuloides)*

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Abstract

As atmospheric CO$_2$ levels rise, temperate and boreal forests in the Northern Hemisphere are gaining importance as carbon sinks. Quantification of that role, however, has been difficult due to the confounding effects of climate change. Recent large-scale experiments with quaking aspen *(Populus tremuloides)*, a dominant species in many northern forest ecosystems, indicate that elevated CO$_2$ levels can enhance net primary production. Field studies also reveal that droughts contribute to extensive aspen mortality. To complement this work, we analyzed how the growth of wild aspen clones in Wisconsin has responded to historical shifts in CO$_2$ and climate, accounting for age, genotype (microsatellite heterozygosity), and other factors. Aspen growth has increased an average of 53% over the past five decades, primarily in response to the 19.2% rise in ambient CO$_2$ levels. CO$_2$-induced growth is particularly enhanced during periods of high moisture availability. The analysis accounts for the highly nonlinear changes in growth rate with age, and is unaffected by sex or location sampled. Growth also increases with individual heterozygosity, but this heterozygote advantage has not changed with rising levels of CO$_2$ or moisture. Thus, increases in future growth predicted from previous large-scale, common-garden work are already evident in this abundant and ecologically important tree species. Owing to aspen’s role as a foundation species in many North American forest ecosystems, CO$_2$-stimulated growth is likely to have repercussions for numerous associated species and ecosystem processes.

Keywords: climate change, CO$_2$ fertilization, heterozygosity-fitness correlation, northern hardwood forests, tree-rings

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Introduction

Forests cover approximately 30% of earth’s terrestrial surface and play critically important roles regulating local environmental and climatic conditions and sequestering greenhouse gases linked to global climate change (Bonan, 2008). Forests of the Northern Hemisphere are particular global sinks for CO$_2$ and their role has increased in recent decades (Myeni et al., 2001; Boisvenue & Running, 2006; but see Stephens et al., 2007). Despite this importance, we still lack a complete picture of how historic increases in atmospheric CO$_2$ and changes in climate are together affecting tree growth, productivity, and thus their capacity to sequester further CO$_2$.

Quaking aspen *(Populus tremuloides* Michx.; here referred to simply as aspen) is a dominant forest type in north-temperate, montane, and boreal regions of North America. The most widely distributed tree species on the continent, *P. tremuloides* is the dominant species on about 17 million ha of Canadian forest (Peterson & Peterson, 1992); within Wisconsin, USA, the location of the present study, it covers ca. 1 million ha (http://www.dnr.state.wi.us/forestry/usesof/aspen.htm). Aspen and related poplars are thus quintessential foundation species (Ellison et al., 2005), shaping the structure and function of the communities and ecosystems in which they occur (Whitham et al., 2006; Schweitzer et al., 2008; Madritch et al., 2009).

In addition to its ecological importance in native forests, *Populus* shows strong responses to enriched CO$_2$ in greenhouse (e.g. Lindroth et al., 2001) and open-top chamber (e.g. Curtis et al., 2000) studies. Work
at the Aspen FACE site in northern Wisconsin, USA, has sought to assess the response of aspen genotypes to CO₂ and ozone enrichment (Karnosky et al., 2003), while parallel work at POPFACE in Italy has evaluated responses of three species of *Populus* to CO₂ (Wittig et al., 2005). Both studies document enhanced primary production in trees exposed to enriched CO₂ in the Aspen FACE experiment, for example, elevating CO₂ by 55% increased aspen growth rates by 40% (Karnosky et al., 2003). These results resemble those of other temperate forest FACE studies, showing that CO₂ enhances net primary production across a broad range of productivity levels (Norby et al., 2005).

Our ability to infer how carbon sequestration in forests will respond to higher CO₂ levels is limited, however, by the nature and design of fumigation experiments. The FACE studies have been relatively short-term (<10 years) and focus on forests of young to intermediate age. They also can incorporate only a limited number of relevant and potentially interacting environmental factors (e.g. soil nutrient and water availability, temperature, ozone, etc.). Finally, CO₂ augmentation studies do not identify how past changes in CO₂ and climate and their interactions with other environmental variables may together influence tree growth under competitive field conditions. Research is needed to investigate how tree species respond to elevated CO₂ levels at larger scales and over longer time periods.

To complement recent experimental and prospective FACE studies, we employed a retrospective approach, measuring tree rings and employing multivariate statistical analyses to assess how growth patterns in quaking aspen have already responded to historical shifts in CO₂ and climate. Because genetically distinct aspen clones differ in their growth responses to experimentally elevated CO₂ (Wang et al., 2000; McDonald et al., 2002) and because growth rates in aspen are known to increase with individual heterozygosity (Jelsinski, 1993; Mitton & Grant, 1996), we also investigated how individual heterozygosity affects aspen's responses to CO₂ and climate. This task was complicated not only because growth rates vary among genotypes, and change as trees age, but also because both climates and CO₂ levels are changing, particularly in the north-temperate and boreal regions of North America (Cayan et al., 2001). Ever since the study by LaMarche et al. (1984) first purported to observe increased tree growth rates in response to rising CO₂ levels, a major difficulty has been to determine whether the increases reflect increasing CO₂ or changes in climate. Such work is further complicated by variation in other factors, including age, site (aspect, slope, soil, elevation), and morphology (strip-bark vs. continuous cambium).

Annual rings in aspen are more difficult to measure than those of conifers, which have been the main focus of research into the effects of past increases in CO₂ on tree growth. Nevertheless, aspens are well-suited for this work both because the species is known to respond strongly to augmented CO₂ and moisture (Fralish & Loucks, 1975; Girardin & Tardif, 2005), and because its clonal growth form allows one to measure multiple ramets (trees) within each genet (clone). In addition, *P. trichocarpa* is the first tree species to have its genome sequenced (Tuskan et al., 2006), providing an abundance of genomic information with which to assess individual variation.

Given that this major forest species shows strong, genotype-specific responses to elevated CO₂, in this work we ask whether past increases in ambient CO₂ have affected the growth of quaking aspen, and how rising CO₂ may have interacted with moisture availability. We further ask whether age, sex, and genetic variation play a role in this species’ response to rising CO₂. Although the study reported here encompasses only a small portion of the total range of this broad-ranged species, the use of wild-grown trees from various soil types, weather patterns, and associated vegetation, means that inferences from this work should apply broadly.

**Materials and methods**

**Field collections and sampling**

We collected branch samples from 919 trees after leaves dropped in the fall. These samples represent 189 genets (clones; 5 trees clone⁻¹) at 11 sites distributed among three regions in Wisconsin, USA (Fig. 1, Table S1 in Supporting Information online). We sampled trees 5–76 years old (mean: 22.5) from forests dominated by aspen and birch, noting GPS coordinates for each clone. Our sites represent second-growth, unmanaged forests south of the areas defoliated by forest tent caterpillars in 1980–1982, 1989–1990, and 2001–2002. The sampled clones were separated by tens to hundreds of meters of other vegetation. We only sampled neighboring clones if they were of different sexes, and we confirmed the unique identity of each genet by comparing all multilocus genotypes within each population. We used a 10 m pole pruner to collect one branch from one tree (ramet) of each clone and then collected buds for DNA extraction. We recorded sex as male, female, or non-reproductive (rare for full-grown trees). We also collected increment cores and measured diameters at breast height from the five largest trees (ramets) in each clone (genet), avoiding any obviously damaged or suppressed trees, in order to minimize potential
shading effects. Aboveground growth as measured by diameter at breast height, which directly reflects annual rings, is very highly correlated with total biomass in aspen (Bond-Lamberty et al., 2002).

Tree ring analysis

Cores were dried at 100 °C for ca. 48 h, then glued into grooves in plywood so the vessels were vertical, sanded with increasingly fine sandpaper (to 400 grit), and stained with Fehling’s solution. Ring widths were measured under ×7–30 magnification on a binocular dissecting microscope with a crosshair reticule, using a data logger that recorded increments to 0.001 mm. For off-center cores, the distance to the center and number of rings were estimated by overlaying a plastic sheet printed with circles of different radii so that the arc segments of the rings present matched a circle on the plastic, and dividing the radius of this circle by the mean of the last four counted rings, to estimate the total age of the stem. Graphs of annual ring widths for all five ramets of a clone were plotted together and examined for anomalies that would indicate any error in identifying a ring boundary; when any errors were suspected, the cores were re-examined to correct or confirm the measurements.

Microsatellite analysis

DNA purification, polymerase chain reaction (PCR) reactions, and capillary electrophoresis are described elsewhere (Cole, 2005). Microsatellite amplifications were conducted on 16 loci, using previously developed primers (van der Schoot et al., 2001; Tuskan et al., 2004, http://www.ornl.gov/sci/ipgc/ssr-resource.htm). All loci were polymorphic, and the level of heterozygosity observed ($H_{obs}$) was calculated as the proportion of loci for which each individual was heterozygous.
CO₂ and climate

Atmospheric CO₂ data were obtained from the Mauna Loa observatory records (http://cdiac.esd.ornl.gov/trends/CO2/sio-info.htm), which have been recorded monthly since 1958; for each year, we used the mean of the April to September values, which span the growing season. We obtained moisture information from the North American summer values of the Palmer Drought Severity Index (PDSI) (ftp.ngdc.noaa.gov/paleo/drought/pdsi2004/data-by-gridpoint) for locations No. 198 (92.5°W, 45.0°N), 207 (90.0°W, 45.0°N), and 208 (90.0°W, 42.8°42.5°N). Mean annual precipitation at these sites ranges from 81 to 86 cm (http://www.crh.noaa.gov/mkx/climate/wipcpn.gif). Because these sites are roughly equidistant between these three locations, we used the mean PDSI value calculated from the two adjacent PDSI sites. Thus, the central Lakes Coulee population (91.16°W, 44.16°N) used the mean PDSI values from sites 198 and 207. PDSI values for these three regions are highly similar (data not shown). Temperature during the growing season was not explicitly included in the model (except indirectly, as it contributes to the Palmer Drought Severity Index), since summer temperatures during the 1950–2000 period have not changed significantly (Feng & Hu, 2004).

We were able to analyze the separate and joint effects of CO₂ and moisture (PDSI) on tree growth because they have varied in different ways in recent decades. That is, while CO₂ has increased monotonically, moisture (PDSI) has varied widely among years. Thus, consecutive years could experience an increase in CO₂ but either a decrease or an increase in PDSI. In addition, over time, years occurred with the same PDSI but very different CO₂ levels (the frequency of observations at different levels of and PDSI is shown in Fig. S1). This asynchrony between the PDSI and CO₂ trends produced a statistical independence that gave the model statistical strength.

Statistical model and analysis

Our modeling process sought to determine the independent and interactive effects of each of the factors listed above. As a response variable, we used (ring width)¹⁄₂ to stabilize the small amount of heteroskedasticity in the ring width measures. We modeled this response for each ramet (tree) in a nonlinear, mixed effects model, i.e. one that allows for factors to have both fixed effects (having the same magnitude for all individuals) as well as random effects, distributed as random variates among individuals. In this way, we accounted for the strong and highly nonlinear effect of age on growth, as well as variation arising from both genetic and environmental sources (e.g. soil type, slope, etc.). Thus, ring width is composed of an overall value common to all measurements, with an adjustment for each genet and for each ramet within that genet. This produced an age-specific growth curve (Fig. 2). The model then analyzes how this age-specific growth would be affected by each of the other factors analyzed, alone and in combination. The final form of the statistical model was developed primarily through likelihood ratio testing of model components. Competing model formulations were also compared using the Akaike Information Criterion (AIC), the Bayesian Information Criterion (BIC), and examination of model residuals. We further tested the model by randomly subdividing the dataset into ‘training’ and ‘validation’ subsets, and estimating the residual error between the subsets as well as the AIC values for different levels of model complexity; this random subdivision was replicated 10 times (details and results in online Supporting Information). The predictive strength of the model, as measured by these criteria, greatly increased when the model included higher-order functions of CO₂ and PDSI, as well as their interactions. The model was also substantially strengthened by incorporating an autocorrelation function [AR(3)], which accounts for the effects of changing environmental conditions persisting through subsequent years.

The general form of the model used is

\[ y_{ij} = f(H_{obs}, \text{CO}_2, \text{PDSI}, \theta) + g(Age, x, \beta, \gamma, \delta) + h(sex, region, \theta) + e_{ij}, \]

Fig. 2 Age-specific growth of quaking aspen trees. Highly nonlinear growth patterns are shown in this graph of ring width (square root of increment in mm) vs. tree (ramet) age (years). Data points are mean values for all trees of each age, uncorrected for any other factors; the dashed line shows the model function at mean values for other factors.
where \( y_{ij} \) refers to growth of an individual ramet (tree) \( i \) in genet \( j \) during each year, and \( \varepsilon_{ij} \) is random error associated with that individual at the specified \( \text{Age} \). The \( f \) and \( g \) refer to functions involving \( \text{Age} \) and \( (\text{CO}_2, \text{PDSI}, \text{and } H_{\text{obs}}) \), all of which proved to be significant; the \( h \) refers to functions involving \( \text{sex} \) and \( \text{region} \) (of Wisconsin), which proved to be nonsignificant. The model is a fourth-degree polynomial with linear, quadratic, cubic, and quartic terms, and interaction effects between \( \text{CO}_2 \) and PDSI, described more fully in the Appendix A (we did not find evidence of other significant interactions). Other factors known to affect aspen growth (ozone, nitrogen deposition, herbivory) could be excluded from the analysis for \textit{a priori} reasons discussed in the online Supporting Information, along with further details of the analysis. Preliminary linear regression analysis identified the significant first-order effects reported here, but the mixed-effects model approach provides more accurate expression of nonlinear response patterns, components of variation, and predictor effects.

Overall growth increase during 1958–2003 (the period for which \( \text{CO}_2 \) data are available) was estimated using ring widths, their square roots, and the model described above. In this latter case, age was held constant, and default values for categorical variables ‘sex’ and ‘region’ were used, along with mean values for \( H_{\text{obs}} \) and PDSI; the model was then evaluated for mean summer \( \text{CO}_2 \) levels of 1958 and 2003 (316 and 376 ppm, respectively).

Results

Aspen growth rates have accelerated markedly since 1935 (Fig. 3). Four factors in the model significantly affected growth: age, individual heterozygosity \( (H_{\text{obs}}) \), \( \text{CO}_2 \), and moisture (PDSI).

Growth rate varies strongly and nonlinearly with age, peaking at about age 9 and declining thereafter (Fig. 2). Age-specific ring width increased over time, and the greatest increase occurred for relatively young trees, so that young trees grow faster in recent years than did young trees several decades ago. For example, during the past five decades, growth of trees 11–20 years old increased by 60% (Table S2). The overall increase shown in Fig. 3 cannot be explained by age of the trees sampled, since the average age of the trees increases during most of the time period sampled (data not shown). Estimates of changes in growth rates for older trees are strongly influenced by the small number of trees present at the beginning of the period studied [e.g., only one tree (ramet) was 30 years old by 1960; Table S2].

Clonal genotype (specifically individual-level heterozygosity, \( H_{\text{obs}} \)) has the simplest effect on aspen growth rate (Fig. 4). As in previous studies with isozymes in aspen (Mitton & Grant, 1980; Jelinski, 1993; Liu & Furnier, 1993), microsatellite loci reveal high levels of genetic diversity at the population level, as well as individual heterozygosity \( (H_{\text{obs}}; \text{mean } = 0.410; \text{range: } 0.125–0.688) \). Individual heterozygosity had a simple, linear effect enhancing tree growth in these natural stands \( (P = 0.038) \). While the data show high levels of

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**Fig. 3** Mean ring width over time. The overall increase in ring width is evident even in the raw data, as are the effects of dry (e.g. 1988) and wet (e.g. 1993) years. The number of trees included in the data set increases with time, as does the mean age of trees in the sample.

**Fig. 4** Individual heterozygosity \( (H_{\text{obs}}) \) increases growth rates. Data points are mean ring widths (square root transform) for each of 189 aspen genets, averaged across all ages and years for all ramets. The solid line indicates the significant \( (P = 0.0376) \) linear effect of \( H_{\text{obs}} \) on aspen growth.
variability arise from other factors (including age, CO2 level, and moisture, as well as other factors not measured), the effect of Hobs was significant in all versions of the model tested. We found no evidence of interactions between heterozygosity and the other factors affecting growth.

The effects of rising CO2 are especially strong as reflected in Figs 5 and 6, and the model coefficients (Table 1). Note that although many λ coefficients are small, they are multiplied by one or both factors in the model raised to a second, third, or fourth power, so their combination makes a major and significant contribution to the model (Fig. S6). Growth responses to CO2 interact strongly with moisture (P<0.001, Table 1), reflecting greater growth increases at higher moisture levels. As shown in Fig. 5, rising CO2 causes ring width to increase at all moisture levels, apparently resulting from improved water use efficiency, but the overall increase shown in Fig. 3 results from historical increases in both CO2 and water availability. For a tree of average age (22 years in this set), the effect of rising CO2 has been to increase ring width by about 53%. This change corresponds to a 19.2% increase in ambient CO2 levels during the growing season, from 315.8 μL L−1 in 1958 (when CO2 records began) to 376.4 μL L−1 in 2003, a period that spans the ages of all but 12 of the 919 trees studied.

While the effects of individual factors are quantified in Table 1, their joint effects may be better conveyed graphically. For example, Fig. 6a illustrates the same historical record of average ring width over time shown in Fig. 5, along with a curve illustrating the ring width that would be predicted by a model that included only the age of the trees sampled at each time. Figure 6b illustrates the ring width that would be predicted when the suite of minor factors is added (i.e. sex, region, and Hobs) – factors that do not change over time. Since these constant factors do not increase the predictive power of the model, their addition increases the AIC value slightly. Figure 6c shows the dramatic increase in predictive power when moisture (PDSI) is added to the model– an effect particularly noticeable in extremely dry and wet years (see Supporting Information Fig. S3). The model illustrated in Fig. 6c also includes the nonlinear functions of PDSI, both singly and interacting with CO2. Finally, Fig. 6d shows the improvement arising from the complete model, which includes CO2 along with the factors previously mentioned. Marked declines in the AIC values, inset in each panel, reflect the substantial improvements in model strength when PDSI and CO2 are added; relative importance of different components of the model are also indicated in Fig. S6.

As might be expected in such longitudinal data, we observed significant correlation over time between the residuals of ramets (trees). We found evidence that a degree 3 autoregressive process (described further in the Appendix A) was a satisfactory model for the autocorrelation between observations within ramets. Figure S2 shows the autocorrelation patterns for three models of the error correlation process: AR(3), AR(1), and a model assuming no correlation between the errors within a ramet. The figure shows the substantial reduction in correlation between the residuals for using the AR(3) model formulation. These differences were tested with likelihood ratio tests, and the AR(3) formu-
lation was significantly better than lower order autoregressive structures.

Neither sex nor region had a significant effect on growth. We did find that the amount of variation among genets was about three times greater than among ramets. This result reflects both the genetic differences among clones (genets) as well as the greater environmental uniformity found within clones relative to site and environmental differences that exist among dispersed clones. Table 1 shows several nonsignificant parameter estimates (θ values) from the complex polynomial function for PDSI and CO2. We included nonsignificant terms in the polynomial function when these terms belong to a parameter set that is statistically significant by likelihood ratio testing. For example, the parameter estimates for degree 2 × degree 4 interactions for PDSI and CO2 are not statistically significant individually (P values 0.1182 and 0.4874, respectively; see Table 1), but a likelihood ratio test for including this set of parameters gave a P-value of 0.06, sufficiently small to suggest including these terms in the final model.

Discussion

Increases in both CO2 and precipitation have contributed to the higher growth rates we observe in Wisconsin aspen (Figs 3, 5 and 6, Fig. S6). Although strong drought conditions in some years (e.g. 1977, 1988) reduced aspen growth, precipitation in Wisconsin has generally increased in recent decades (Feng & Hu, 2004; Fig. S3). This increase contrasts with the prolonged drought in the prairie provinces and northern plains states that has contributed to widespread aspen mortality in that region (Hogg et al., 2005) and to the recent, acute drought that has contributed to sudden aspen decline (SAD) in the central Intermountain West (Worrall et al., 2008). The improved growth under low moisture conditions shown here may reflect higher water use efficiencies in CO2-enriched atmospheres (Ceulemans & Mousseau, 1994).

The substantial, CO2-mediated growth increase identified in this work stands in contrast to previous studies that have sought to document how rising atmospheric
Table 1  Fixed and random effects on growth of 919 ramets representing 189 genets of *Populus tremuloides*

<table>
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<th>Parameter</th>
<th>Value</th>
<th>Error</th>
<th>P-value</th>
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$\theta$ coefficients from the nonlinear mixed-effects model account for CO$_2$, moisture, heterozygosity, and their interactions; and for non-significant factors (region, sex). Coefficients $\alpha$, $\beta$, $\gamma$, and $\delta$ are used in function $g$ describing the effect of age on growth. Coefficients $\theta$ are subscripted to indicate which factors they refer to: $R1$ and $R2$ refer to region 1 (north) and region 2 (central), compared with region 3 (south); SM and SF refer to male and female, compared with nonreproductive; and $H1$ refers to the effect of individual heterozygosity. $C1$-$C4$ are first- through fourth-order effects of CO$_2$, $P1$-$P4$ are first- through fourth-order effects of precipitation. Subscripts with C-P refer to the interaction between CO$_2$ and precipitation, with numerals indicating the level of the interactions. Non-significant parameters are retained when their interaction parameters are significant. Note that while many $\theta$ coefficients are very small, in the model they are multiplied by their factor (e.g., CO$_2$ level) raised to a second, third, or fourth power.

CO$_2$ levels may affect tree growth (summarized by Huang et al., 2007). Only a few studies have attempted to incorporate effects of both climate and rising CO$_2$ levels on tree growth in natural systems. Among these, work by Voelker et al. (2006), analyzing the effects of climate (PDSI), tree age, and CO$_2$ on oaks and pines of the central United States, found strong evidence that the growth of these trees has also increased with rising CO$_2$, and these effects decline with age. These results are similar to those we found in *P. tremuloides*, although the linear regression model they used did not have the same power to quantify the interactions among these factors or how those interactions changed over time. Several tree species in Europe (Körner et al., 2005) and in the southern United States exposed to elevated CO$_2$ show little increase in aboveground growth, perhaps due to greater allocation to fine roots (Norby et al., 2004) or higher rates of respiration (Feeley et al., 2007). However, aspen trees grown in the FACE experiment in northern Wisconsin, which show a marked growth response to CO$_2$ enrichment, have little or no change in their size-specific allocation to roots as a result of the added CO$_2$ (King et al., 2005). Also, while the length of the growing season affects aspen growth (Yu et al., 2001), climate records indicate that the growing season in Wisconsin has increased only 2.5–5 days since 1951 (Feng & Hu, 2004), a change too small to account for the large growth increases we have observed.

Most efforts to determine how rising CO$_2$ affects tree growth have focused on conifers in the western United States (e.g., LaMarche et al., 1984). Such results tend to be equivocal, possibly because annual increments are also influenced by elevation and cambium morphology (strip- or continuous-bark), or because they are hampered by incomplete CO$_2$ and weather records (LaMarche et al., 1984; Graumlich, 1991; Graybill & Idso, 1993; Jacoby & D’Arrigo, 1997; Soule & Knapp, 2006). Other studies implicating a role for CO$_2$ have used models to predict the response of tree growth to changes in climate, and then noted elevated rates of growth in recent decades that are difficult to explain in terms of the climate models (West et al., 1993; Knapp et al., 2001; Wang et al., 2006).

By including a 3-year autocorrelation function, our model incorporates the effects of growth in each of the 3 previous years on growth in the current year (Fig. S2). Although we explored using mean PDSI values from previous years, we found the model to be stronger if it explicitly represented the effect of previous year’s growth using the AR(3) autocorrelation function instead. Leonelli et al. (2008), who also found that aspen growth increases with moisture, concluded that the strongest effect of moisture on growth came from moisture during the previous summer rather than the current year. Their analysis was based on monthly weather records for the current and previous year rather than on yearly means. We used mean values for moisture through the growing season in order to incorporate weather, CO$_2$ age, genotype, and higher-order interactions; analyses including monthly moisture were too complex. The positive response to
moisture reported here for aspen is quite different than that of *Pinus ponderosa*, which showed increased growth in response to rising CO₂ on dry but not moist sites (Soule & Knapp, 2006). Other studies of aspen, balsam poplar, and birch suggest that broadleaved species may be generally more sensitive to moisture and temperature than coniferous trees (Girardin & Tardif, 2005).

The gradual reduction of CO₂-mediated growth enhancement (the plateau seen in Fig. 5a) could reflect a saturating growth response to rising CO₂ levels, although trees grown under elevated CO₂ at the aspen FACE site have not shown saturation (King *et al.*, 2005; Kubiske *et al.*, 2007). Alternatively, growth could become limited by some parallel, external factor such as available N (Luo *et al.*, 2004) or increased exposure to O₃. However, aspen trees growing at the Wisconsin FACE site, as well as other species there and at other forest FACE sites, have responded to elevated CO₂ by increasing their uptake of N, rather than showing progressive N-limitation (Finzi *et al.*, 2007). Addition of ozone to the CO₂ enrichment experiments at Aspen-FACE has shown that O₃ reduces CO₂-induced growth enhancement (King *et al.*, 2005; Kubiske *et al.*, 2006), especially under dry, high-light conditions. Ozone levels generally follow a gradient that is low in northwest Wisconsin and high in the southeast (http://www.epa.gov/oar/oaqps/uscanpl.pdf, dnr.wi.gov/org/aw/air/monitor/ozonetrends.html). In summer, ozone can reach levels that affect aspen growth (Berang *et al.*, 1991; King *et al.*, 2005). Although not statistically significant, the marginally lower growth rates of aspen trees in southern Wisconsin relative to northern Wisconsin may reflect slightly higher ozone exposures there.

Individual heterozygosity (*H*<sub>obs</sub>) had a modest but significant effect on growth rate, even though addition of *H*<sub>obs</sub> did not improve the predictive value of the model, since it (like the nonsignificant factors sex and region) does not change over time. Thus, we found no significant interactions between *H*<sub>obs</sub> and those factors that did change over time, i.e. age, CO₂, and moisture. Previous isozyme studies in wild aspen that account for environmental factors such as age and elevation also found higher growth in more heterozygous individuals (Mitton & Grant, 1980; Jelinski, 1993). Although it is possible that heterozygosity per se at (or near) isozyme loci might increase growth, our finding that microsatellite *H*<sub>obs</sub> enhances growth (Fig. 4) suggests instead that both sets of markers reflect overall levels of inbreeding across substantial portions of the genome (‘associative overdominance’ – Keller & Waller, 2002). Although aspen is dioecious and cannot self-fertilize, localized pollen and seed dispersal could cause enough biparental inbreeding in some individuals for inbreeding effects to be expressed.

The importance of the interactive effects (CO₂ × PDSI) presented here indicate that the ‘fertilization’ effect of rising CO₂ is strongly contingent on precipitation. The higher-order terms must be viewed with some caution, since they can be influenced by vagaries of the particular CO₂ × PDSI values provided historically (Fig. S1); furthermore, they can also reflect the interaction between the growth-promoting effects of moisture and the growth-limiting effects of cloudy days and cool temperatures, which can markedly influence aspen’s response to elevated CO₂ (Kubiske *et al.*, 2006). Even at the linear level, though (Fig. 5b), their interaction indicates that predictions of the effects of future CO₂ increases must be conditioned by moisture availability – a conclusion underscored by the extensive mortality of aspens in western North America, which have experienced the same CO₂ increase as the trees studied here. These results also suggest the importance of extending this kind of analysis to cover greater changes in moisture, over greater geographical and temporal scales. Because both age and moisture influence responses to CO₂ enrichment, large-scale common-garden work (such as the Aspen FACE project) should be extended to cover greater ranges of both climatic (especially moisture) flux as well as tree age, to provide more robust predictions of how aspen-dominated forests will respond to future CO₂ increases. Finally, the magnitude of the growth increase uncovered by this analysis raises the question of how much other major forest species have responded to the joint effects of long-term changes in CO₂ and precipitation.

Quaking aspen constitutes a major foundation species in forest ecosystems throughout much of North America, influencing plant, herbivore, and decomposer communities. The species contributes substantially to the productivity of northern temperate forests, which has increased over the past half-century (Myeni *et al.*, 2001; Boisvenue & Running, 2006). Several indirect estimates of forest growth rates in the eastern United States suggest that this increase arises primarily from changes in land use (e.g. afforestation), while the effects of rising CO₂ have been modest (Schimel *et al.*, 2000) or virtually nil (Caspersion *et al.*, 2000). The more direct analysis presented here reveals that rising concentrations of CO₂ during the past five decades have already strongly influenced growth rates of this major component of North American forests, especially under high-moisture conditions.

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Appendix A: statistical model development

We analyzed the data using a nonlinear mixed effects model that accounted for the five samples from each genet, nonlinear relationships between age and growth rate, and the gradual increase in average tree age that occurs toward the end of the sampled time period. This model accommodates factors that are extrinsic or intrinsic, and fixed or varying, as well as autocorrelation among years. Analyzing growth patterns via individual annual rings allows us to remove the confounding effects of age-specific changes in growth when exploring interannual changes in the effects of genotype, environment, and their interactions.

The model accommodates the inherently nonlinear relationship between age and ring width characteristic of trees as well as the multiple samples (five) from each genet. In this model, the response variable $y_{ij}$ is (ring width)$^{1/2}$ (the transformation stabilizes heteroskedasticity in the ring width measures). The statistical model used is

$$y_{ij} = f(H_{obs}, CO_2, PDSI, \theta) + g(Age, x, \beta, \gamma, \delta) + h(sex, region, \theta) + e_{ij},$$

where $y_{ij}$ refers to (ring width)$^{1/2}$ for an individual ramet (tree) $i$ in genet (clone) $j$ during each year, and $e_{ij}$ is random error associated with that individual at the specified Age, assumed to be a random variable with mean zero and standard deviation $\sigma$. $H_{obs}$ is the observed heterozygosity as described above.

This function is a fourth-degree polynomial with linear, quadratic, cubic, and quartic terms, and interaction effects between $CO_2$ and PDSI. We did not find evidence of other significant interactions. Higher-degree terms were included if statistically significant by likelihood ratio testing, and lower order terms were dropped if significant by likelihood ratio testing, and lower order terms were dropped if not significant by likelihood ratio testing.


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down, varying among stems. Thus ring width is composed of an overall value common to all measurements (\( \delta \)), with adjustment for each genet (\( d_j \)) and each ramet (\( d_r \)). Similarly, \( z' = z + \delta \), where \( z \) represents the fixed effect for all observations and \( \delta \) represents variation among genet values for the \( z \) parameter. These \( z' \) parameters control the amount of curvature in the age-specific growth curve. In our investigation, we did not find significant variation in the \( z \) parameter among ramets within genets. The random effects defined here for each ramet and genet can be assumed to have a general correlation pattern that includes independence as a possible structure.

The other parameters in this model, \( \beta \) and \( \gamma \), are fixed effects that hold for all observations in the study. In other words, the model says that the growth pattern across age follows the same basic pattern with respect to the parameters controlled by \( \beta \) and \( \gamma \), but variation among ramets and genets determines how the \( z \) and \( \delta \) parameters affect individual growth curves.

Function \( h \) accounts for the effects of sex of each genet (staminate, pistillate, or nonreproductive) and the region where each population was located (north, central, or south Wisconsin, listed in Table S1), and is defined as

\[
h(\text{sex, region}, \theta) = \theta_{SM} \times SXM + \theta_{SF} \times SXF + \theta_{R1} \times \text{REG1} + \theta_{R2} \times \text{REG2}
\]

where variables \( SXM \) and \( SXF \) are indicator variables taking values of 0 or 1 depending on the sex of the genet: \( SXM = 1 \) for males, else 0, and \( SXF = 1 \) for females, else 0. Thus the few nonreproductive genets have values of 0 for both indicators, and the values of \( \theta_{SM} \) and \( \theta_{SF} \) reflect gender-specific growth rates relative to those of nonreproductive trees. Similar indicator variables represent regions: \( \text{REG1} = 1 \) for Region 1 (southwestern Wisconsin), else 0, and \( \text{REG2} = 1 \) for Region 2 (central Wisconsin), else 0. These \( \theta \) coefficients measure the impact of region on ring width. For example, \( \theta_{R1} \) measures the impact of growing in Region 1 compared with Region 3 (northern Wisconsin), after adjusting for the other terms in the model. A similar interpretation applies for \( \theta_{R2} \), the impact arising from growing in region 2 compared with region 3.

Because ring width observations are made over time, they form a time series record. In many applications similar to this, and in these data, we observe correlations between observations taken near each other in time. For this reason, we have modified the error correlation structure to allow correlation between ring width measurements. Several correlation structures were tested, and a third-order autoregressive process [AR(3)] seems to give a reasonable representation of the correlation pattern. An AR(3) correlation structure implies that for a particular ramet, the ring width error \( e_t \), at time \( t \), can be written as

\[
e_t = \varphi_1 e_{t-1} + \varphi_2 e_{t-2} + \varphi_3 e_{t-3} + \epsilon_t
\]

where \( \epsilon_t \) is a pure noise term with mean zero, and independent of previous observations. The data suggest such a structure is reasonable; in turn this means errors in previous periods carry some information about the error term in the current period. In terms of aspen biology, this suggests several possibilities, one being that growing conditions during one year have effects on growth for several subsequent years, from the storage of photosynthates, production of vegetative buds, etc.

**Supporting Information**

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

**Figure S1.** Sunflower plot of frequency of observations.

**Figure S2.** Residuals from autoregression functions.

**Figure S3.** Annual moisture changes over time in the region studied.

**Figure S4.** Age-specific growth rates over time.

**Figure S5.** Model residuals by year for different age groups.

**Figure S6.** Relative strength of different versions of the model.

Table S1. Sample locations and sizes.

Table S2. Mean ring widths for trees of different ages over time.

Table S3. Model testing by subdivision into ‘training’ and ‘validation’ subsets.

Appendix S1. Supplementary analytical methods and modeling information.

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