New Phytologist

Research

Can elevated CO_2 and ozone shift the genetic composition of aspen (*Populus tremuloides*) stands?

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Received: *26 November 2012*
Accepted: *18 December 2012*

New Phytologist (2013)
doi: 10.1111/nph.12153

Key words: Bayesian, CO_2, FACE, growth, mortality, ozone, *Populus tremuloides* (aspen), selection.

Summary

- The world's forests are currently exposed to increasing concentrations of carbon dioxide (CO_2) and ozone (O_3). Both pollutants can potentially exert a selective effect on plant populations. This, in turn, may lead to changes in ecosystem properties, such as carbon sequestration. Here, we report how elevated CO_2 and O_3 affect the genetic composition of a woody plant population via altered survival.
- Using data from the Aspen free-air CO_2 enrichment (FACE) experiment (in which aspen clones were grown in factorial combinations of CO_2 and O_3), we develop a hierarchical Bayesian model of survival. We also examine how survival differences between clones could affect pollutant responses in the next generation.
- Our model predicts that the relative abundance of the tested clones, given equal initial abundance, would shift under either elevated CO_2 or O_3 as a result of changing survival rates. Survival was strongly affected by between-clone differences in growth responses. Selection could noticeably decrease O_3 sensitivity in the next generation, depending on the heritability of growth responses and the distribution of seed production. The response to selection by CO_2, however, is likely to be small.
- Our results suggest that the changing atmospheric composition could shift the genotypic composition and average pollutant responses of tree populations over moderate timescales.

Introduction

Over the next century, forest trees will be subjected to increasingly altered atmospheric composition. By 2100, the concentration of atmospheric carbon dioxide (CO_2) is expected to be between 1.5 and 4 times higher than at the beginning of the century (IPCC, 2007) and at least one-half of the world's forests will also be exposed to elevated ozone (O_3) (Fowler *et al.*, 1999). CO_2 'fertilization' tends to increase growth in woody plants (Gielen *et al.*, 2005; Norby *et al.*, 2005), allowing many forests to serve as carbon sinks. Conversely, O_3 damages the photosynthetic apparatus of leaves and leads to reduced productivity (Dickinson *et al.*, 1991; Isebrands *et al.*, 2001; Kontunen-Soppela *et al.*, 2010). However, genetic variation in response to both pollutants has been detected in many plant taxa, indicating the potential for adaptive responses (Berrang *et al.*, 1989; Dickinson *et al.*, 1991; Heagle *et al.*, 1991; Paakkonen & Holopainen, 1995; Whitfield *et al.*, 1997; Wang *et al.*, 2000; Ziska & Bunce, 2000; Isebrands *et al.*, 2001; Karnosky *et al.*, 2003; Haikio *et al.*, 2009; Nakamura *et al.*, 2011). Such adaptive responses may be important for the maintenance of forest productivity and carbon uptake under future conditions. In addition, the genetic composition of forests affects numerous community and ecosystem properties, including herbivore communities and nutrient cycling (Dickson & Whitham, 1996; Bailey *et al.*, 2006; Whitham *et al.*, 2008; Madritch *et al.*, 2009). In this analysis, we use long-term data from the Aspen free-air CO_2 enrichment (FACE) experiment to ask whether elevated levels of CO_2 and O_3 exert a selective effect on quaking aspen (*Populus tremuloides*).

Many plant species, including aspen, exhibit genetic variation in CO_2 and O_3 responses. O_3 tolerance is heritable (Berrang *et al.*, 1989; Heagle *et al.*, 1991; Whitfield *et al.*, 1997; Lee *et al.*, 2002), and the frequency of tolerant genotypes tends to increase in populations exposed to chronically elevated [O_3] (Berrang *et al.*, 1989; Heagle *et al.*, 1991). Aspen clones known to be sensitive to O_3 exhibit reduced growth and increased mortality as a result of competitive exclusion at high [O_3] (Berrang *et al.*, 1989; Karnosky *et al.*, 2003). However, little is known about how selection for O_3 tolerance is likely to affect the average O_3 response over multiple generations. Similarly, the selective impact of CO_2 has been studied in a number of herbaceous species, but studies in woody plants are rare, and it is unclear whether shifts in community composition will translate into changes in the average CO_2 response in the next generation. Elevated CO_2 has the indirect effect of increasing competition because it increases growth (Steinger *et al.*, 2007), and can disproportionately increase the growth of dominant individuals (McDonald *et al.*, 2002; Hikosaka *et al.*, 2003). Elevated CO_2 may therefore

change the genetic composition of plant populations when growth responses differ between genotypes (Andalo *et al.*, 2001; Kubiske *et al.*, 2007). Selection under elevated CO_2 for traits associated with competitive ability, including size, relative growth rate and seed number, has been observed in some, but not all, herbaceous species studied (Ward *et al.*, 2000; Ziska & Bunce, 2000; Wieneke *et al.*, 2004; Steinger *et al.*, 2007; Nakamura *et al.*, 2011).

The Aspen FACE experiment in Oneida County, Wisconsin (http://aspenface.mtu.edu/) was established to test the impact of elevated CO_2 and O_3 on quaking aspen. Five aspen clones were exposed to four treatments: control, elevated CO_2, elevated O_3 and elevated CO_2 and O_3. McDonald *et al.* (2002) showed that, during the first 4 yr of the Aspen FACE experiment, trees that were taller than their neighbors showed much stronger responses to CO_2. A strong influence of clone identity on growth rate and clone × treatment interactions in growth was observed in the first 3 yr (Isebrands *et al.*, 2001). After 7 yr, clone 42E was emerging as the most successful in elevated CO_2 and clone 8L in elevated O_3, based on an importance index combining both volume growth and survival (Kubiske *et al.*, 2007). In a separate glasshouse experiment, enriched CO_2 increased the magnitude of among-clone variation in aspen growth (Lindroth *et al.*, 2001).

In this study, we make use of the full 12 yr of data from the Aspen FACE experiment to ask:

- Do clone × treatment interactions in survival exist and, if so, will this result in altered rank abundance of clones under elevated [CO_2] and/or [O_3] (Wieneke *et al.*, 2004), given equal initial abundances?
- If such an interaction exists, is it primarily a result of differences in growth responses between clones (Kubiske *et al.*, 2007)?
- Given the observed mortality patterns in these stands, is selection likely to shift the average CO_2 or O_3 response of the next generation?

In order to address these questions, we developed a set of hierarchical Bayesian models of survival to test whether genotype × environment interactions are important for explaining the data and whether differences in growth responses explain the differences in survival. We also calculated the expected change in mean response to CO_2 and O_3 in the next generation, given a range of assumptions about the heritability of the growth response and the equality or inequality of reproductive success.

Materials and Methods

Study species

Trembling aspen (*Populus tremuloides*) is widely distributed in North America (Mitton & Grant, 1996). It reproduces both sexually and asexually, and a large amount of genetic variation exists both within and between populations (Mitton & Grant, 1996). Heritable variation has been documented in multiple ecologically relevant traits, including growth rate and leaf chemistry (Mitton & Grant, 1980; Thomas *et al.*, 1997; Cole *et al.*, 2009). Because *Populus* species are fast growing and easy to propagate vegetatively, they are commonly cultivated for wood pulp and as a potential biofuel, and have become the pre-eminent model organism for woody plant genetics (Tuskan *et al.*, 2003).

The Aspen FACE experiment

The treatments were as follows: Control (360 ppm CO_2, 33–67 ppb O_3), +CO_2 (560 ppm CO_2), +O_3 (1.5 × control) and +CO_2 + O_3. Three FACE rings were exposed to each treatment. One-half of each ring was planted with a mixture of five aspen clones (1 × 1-m^2 spacing), one-quarter with mixed aspen and birch and one-quarter with mixed aspen and maple. Replicated genotypes within a treatment make it possible to distinguish genetic and environmental effects. Details of the experiment were described by Dickson *et al.* (2000). In this and previous analyses, trees from the outer part of the ring, exposed to peak concentrations of gases, were excluded. Trees were planted in 1997 and were measured annually until 2008. In this analysis, we use data from the aspen-only side of each ring, from trees that were alive in 1998 and that were not harvested during the course of the experiment (Supporting Information Table S1). The competitive environment differed in the mixed-species quarters, and the previous year's growth (used as a covariate in some survival models) is lacking for the first year. From these raw data, we calculated actual percentage survival over the 11-yr period (1998–2008) for each of the three replicates. Because the Bayesian analysis focuses on the individual level, we do not separate individuals by ring (block); environmental differences are reflected by variation in growth or mortality not explained by clone or treatment, and will be taken up either in growth effects or the stochastic term of the model (see the following section).

Survival model

A Bayesian framework makes the construction and comparison of non-nested hierarchical models more straightforward than do classical approaches (Clark, 2005, 2007). First, we represent survival data as an $n \times T$ matrix, S, in which there are n individuals and T years of observations. $S_{it} = 1$ if individual i is alive in year t, and zero if it is dead; thus, S_{it} functions as an indicator random variable. For an individual alive in year $t-1$, θ_{it} is the probability that it will be alive in the next year. The probability of survival depends on a matrix of constant factors X (such as clone or treatment) and a matrix of time-dependent factors W_t (such as age or height). A stochastic component, ε, is included to account for any remaining uncertainty. For full model details, please see Notes S1.

The complete Bayesian model can be expressed as:

$$P(\vec{\beta}, \vec{\alpha}, \sigma | X, W, S) \propto \prod_{i=1}^{n} \prod_{t=1}^{T} [p(S_{it}|\theta_{it}) p(\theta_{it}|X_i\vec{\beta}, W_{it}\vec{\alpha}, \sigma)]$$

$$p(\vec{\beta}|b, \phi) p(\vec{\alpha}|a, \omega) p(\sigma|s_1, s_2)$$

The joint posterior for the parameters we want to estimate is on the left-hand side of the equation. The β parameters indicate the effect of the constant factors on survival, whereas the α parameters indicate the effect of the time-dependent factors. σ is an error parameter. We were particularly interested in whether the inclusion of clone × treatment effects improves the model of survival. The three prior distributions shown on the far right-hand side of the equation represent what was previously known about the relationships described by these parameters; model results were relatively insensitive to the choice of prior (Notes S2). The model was implemented in R (www.r-project.org) using Gibbs sampling (Clark, 2007). We constructed multiple models in order to determine the importance of various covariates for mortality risk (Table S2). We compared them using predictive loss (D_m) which, unlike some other model selection techniques, does not require models to be nested (Gelfand & Ghosh, 1998).

We hypothesized that the inclusion of clone × treatment interaction effects would be important in explaining patterns of mortality and survival – at least in simpler models that do not include individual growth as a covariate. However, we know that the aspen clones differed in their growth responses to different treatments (Isebrands *et al.*, 2001; Kubiske *et al.*, 2007), and that slow growth is a good predictor of mortality for trees growing in competitive situations (Kobe & Coates, 1997; Wyckoff & Clark, 2002; Karnosky *et al.*, 2003; van Mantgem *et al.*, 2003). Therefore, we hypothesized that, if clone × treatment interactions in growth are responsible for clone × treatment interactions in survival, then, when the survival model includes growth as a covariate, the clone × treatment interactions should be less important.

Growth rate

We tested whether relative height growth rate (RGR) differed between treatments and clones, and whether there was a clone × treatment interaction in growth using ANOVA. Block effects were also considered. Tests were conducted for each pollutant treatment vs control, as well as for all treatments together. Analyses were carried out in R.

Changes in clone abundance

In the FACE experiment, the initial numbers of individuals of each clone planted in each treatment differed between clones and between treatments, from an average of 145 individuals of clone 271 to 60–90 individuals of clone 42E, obscuring the effect of mortality on relative abundance (Fig. S1). Therefore, we re-sampled the original growth data by drawing 97 individuals with replacement from each clone × treatment combination. We repeated this process 100 times, and used the best-fit survival model to predict how the final abundance of each clone should change over 11 yr, given equal initial abundances. We used principal component analysis (PCA) to visualize differences in the clonal composition of simulated forests, and calculated the average percentage composition of control, $+CO_2$, $+O_3$ and $+CO_2 + O_3$ stands.

Response to selection

If mortality patterns shift under altered atmospheric conditions, how might this affect the population's responsiveness to CO_2 and O_3 in the next generation? That is, does the pollutant response evolve? The change in the mean trait value of a population from one generation to the next (R) is equal to the heritability of the trait (h^2) multiplied by the selection differential (S) (Gillespie, 2004). The heritability represents the proportion of the trait variation that can be explained by genotype rather than by environmental variation; it thus tends to be higher in controlled environments, such as growth chambers, than in the field. The selection differential represents the difference between the mean trait value for the initial population and the mean trait value for the individuals that survive to breed.

For this analysis, we used the 'fourth-year height growth response', the difference in average fourth-year height between the pollutant treatment (either CO_2 or O_3) and the control treatment, as the trait under selection. We chose this trait because height growth is known to be positively affected by CO_2 and negatively affected by O_3, because differences between clones and treatments in this trait were evident by the fourth year of the experiment (Fig. S1), and because 8 yr of selection elapsed after this point. We then calculated the selection differential in two ways (Notes S3). First, we assumed that all the trees alive in the 12th year would contribute equally to the next generation. In fact, larger trees tend to produce more seed than smaller trees, and tree diameter or basal area is generally considered to be a good predictor of fecundity (Dodd & Silvertown, 2000; LaDeau & Clark, 2001; Gonzalez-Martinez *et al.*, 2006). Thus, in the second calculation, we weighted the height growth response in the 'post-selection' population by the diameter squared.

The heritability of the CO_2 and O_3 height growth response in *P. tremuloides* (or, indeed, any other tree) is unknown. Moreover, the heritability depends on the amount of phenotypic variation produced by variation in the environment. However, we can calculate the expected change in the fourth-year height growth response from one generation to the next for a range of potential heritabilities. We chose a range of heritabilities from 0.05 to 0.6, as this reflects the range of heritabilities observed for growth traits in trees; higher levels are generally only observed for genetically identical individuals in highly controlled environments (Curlin, 1967; Mitton & Grant, 1980; Farmer *et al.*, 1988; Thomas *et al.*, 1997; Lee *et al.*, 2002; Luquez *et al.*, 2008).

Results

Survival and growth

Substantial differences can be observed in the mean response of particular clones to particular treatments (Fig. 1). Clone 271 had the highest survival and clone 259 had the lowest survival in all treatments, and both exhibited reduced survival in all pollutant treatments. Although clone 42E had significantly lower survival than clone 271 or 216 in most treatments, it exhibited 12.8% higher survival in elevated CO_2. Similarly, clone 8L exhibited, on

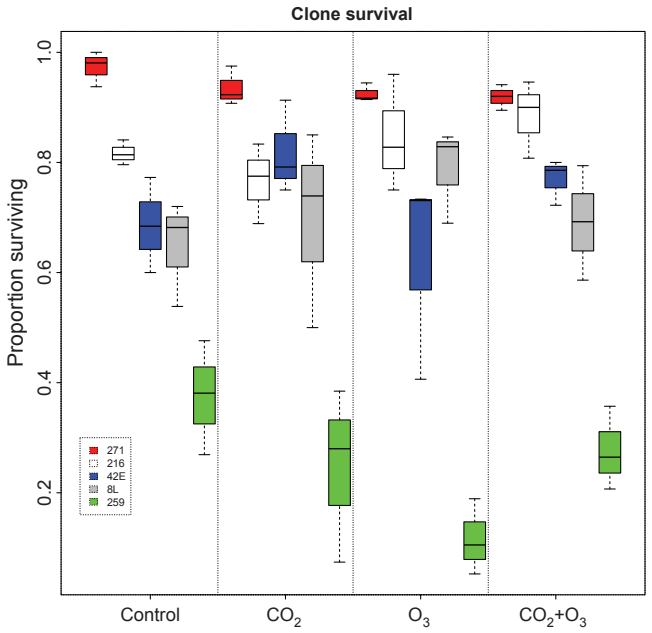

Fig. 1 Observed survival for the five *Populus tremuloides* clones in control, elevated carbon dioxide (CO_2), elevated ozone (O_3) and elevated $CO_2 + O_3$. Whiskers indicate interquartile range for three replicates of each treatment.

average, 14.8% higher survival in elevated O_3, making its survival similar to that of 216, whereas clone 216 performed nearly as well as 271 in the two-pollutant treatment.

The relative height growth rate (RGR) differed between clones and treatments and showed evidence of clone × treatment interactions when treatments were examined separately (Table 1; Fig. 2). Aspen in all three treatments exhibited clone and treatment effects on RGR. Clone × treatment interactions were also significant under elevated CO_2 and elevated O_3, but not in the combined treatment. Block effects were also evident in some treatments, reflecting a productivity gradient across the site. Clone × treatment interactions were not significant when all treatments were considered together, presumably because only a few interactions (e.g. 42E × +CO_2 and 8L × +O_3) were large.

We predicted that clone × treatment interactions would be more important for models in which growth was not considered, and this is indeed what we found. When individual growth was not included as a predictor of survival, the best-fit model was S7a ($D_m = 502.9$), which includes treatment, clone, clone × treatment interactions and age. Among the interaction terms, the largest effects were seen for clone 42E in +CO_2 (β_{13}) and clone 8L in +O_3 (β_{19}), suggesting that these clones should increase their relative abundance under elevated CO_2 or O_3, respectively. However, the inclusion of growth as a covariate improved predictive loss scores substantially, and height increment – growth since the previous year – was a better predictor of survival than current height (Fig. S2; Table S3). The overall best-fit model was S8a ($D_m = 284.3$), which included height increment as well as all the factors in model S7a (Table 2). However, parameter estimates for the influence of age, treatment, clone and interactions were generally small, with confidence intervals widely overlapping zero,

Table 1 ANOVA results for relative height growth rate (RGR) in *Populus tremuloides*

	+CO_2 vs Control				
	df	SS	MS	*F* ratio	*P* ratio
Treatment	1	2.231	2.231	58.203	< 0.0001***
Block	1	0.399	0.399	10.399	0.00132**
Clone	4	11.809	2.952	77.006	< 0.0001***
T × B	1	0.050	0.050	1.301	0.25448
T × C	4	0.884	0.221	5.766	0.00014***
B × C	4	0.199	0.050	1.298	0.26918
T × B × C	4	0.375	0.094	2.442	0.04552*
Residuals	700	26.837	0.038		
	+O_3 vs Control				
	df	SS	MS	*F* ratio	*P* ratio
Treatment	1	0.729	0.729	17.983	< 0.0001***
Block	1	0.428	0.428	10.549	0.00122**
Clone	4	6.655	1.664	41.024	< 0.0001***
T × B	1	0.068	0.068	1.671	0.19667
T × C	4	0.461	0.115	2.841	0.02357*
B × C	4	0.347	0.087	2.141	0.07422
T × B × C	4	0.319	0.080	1.969	0.09764
Residuals	653	26.484	0.041		
	+CO_2 + O_3 vs Control				
	df	SS	MS	*F* ratio	*P* ratio
Treatment	1	0.993	0.993	23.972	< 0.0001***
Block	1	0.000	0.000	0.000	0.9883
Clone	4	13.119	3.280	79.144	< 0.0001***
T × B	1	0.010	0.010	0.246	0.6203
T × C	4	0.225	0.056	1.358	0.2470
B × C	4	0.362	0.090	2.181	0.0696
T × B × C	4	0.355	0.089	2.142	0.0741
Residuals	687	28.469	0.041		

Significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
In interactions: T, treatment; B, block; C, clone.

with the exception of the positive effect of clone 271 and the negative effect of clone 259. Three simpler models including height increment (S14a, S5a and S7b) also performed well, with predictive loss values in the 320s; of these, only S7b included clone × treatment interactions (Table S3). This suggests that clone × treatment interactions in survival can indeed be explained by clone × treatment interactions in growth. Parameter estimates for S14a, S5a and S7b are shown in Table S4.

Abundance

Figure S3 shows the best-fit model survival predictions compared with actual survival over time. The numbers of all aspen clones declined over time as individuals grew and competed. The actual number of individuals usually fell within the 95% confidence interval of the model predictions, although early mortality was sometimes slightly overestimated and late survival slightly underestimated for clone 259. Predicted 12-yr survival (given in

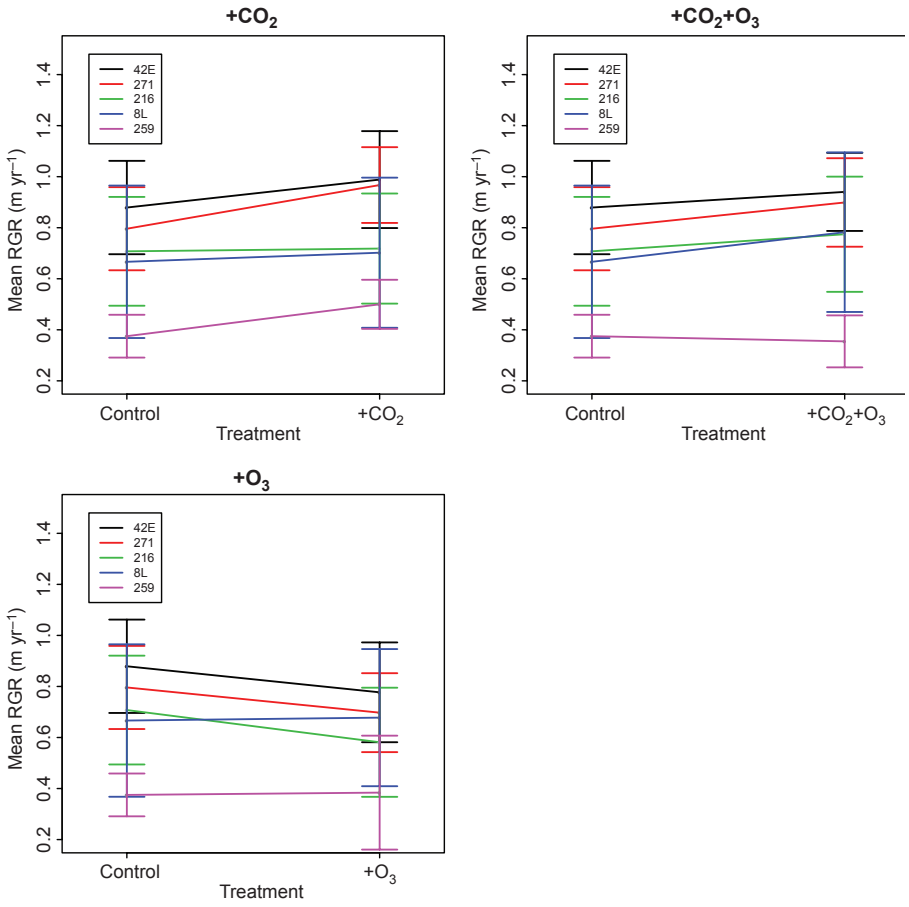

Fig. 2 Mean relative height growth rate (RGR) for each *Populus tremuloides* clone in each treatment. Differences between clones in the slope of the relationship between control and treatment indicates clone × treatment interactions. Bars indicate ± 1SE to each side of the mean.

Table S5) agrees closely with observed survival. Clone 271 always exhibits the highest survival, whereas clone 259 always exhibits the lowest survival. However, clone 42E exhibits increased survival in the $+CO_2$ treatment, clones 8L and 216 exhibit increased survival in the $+O_3$ treatment, and clones 216 and 42E exhibit increased survival in the $+CO_2 + O_3$ treatment.

These differences in survival translate into differences in simulated forest composition (Fig. 3), in some cases resulting in changed expected rank abundances (Table 3). In these simulations, clones each made up 20% of the initial population and populations diverged from this initial equality over time. The clonal compositions of control populations and $+CO_2 + O_3$ populations after 12 yr are relatively similar, as can be seen from their broad overlap in the PCA diagram (Fig. 3). However, $+CO_2$ populations cluster in the lower part of the diagram, indicating a higher proportion of clone 42E relative to control populations. Similarly, $+O_3$ populations cluster at the right side of the diagram, indicating a higher proportion of clones 8L and 216 relative to control populations.

Response to selection

Our calculations indicate that the height growth response to O_3 is likely to be more responsive to selection than is the height growth response to CO_2 (Fig. 4). For heritabilities between 0.05 and 0.6, the response to selection by O_3 in these populations ranged from 0.07 to 0.86, assuming equal fecundity, and from 0.11 to 1.38, assuming that fecundity scales with diameter squared. This means that, rather than the currently observed 7.78% reduction in fourth-year height, at the highest level of heritability one would expect to see only a 6.92% (if offspring production is equal) or 6.4% (if offspring production is unequal) reduction in height as a result of O_3 exposure in the next generation. However, the maximum response to selection by CO_2 was weaker: only 0.24, assuming equal fecundity, and just 0.11, assuming that fecundity scales with diameter squared. This occurs because the clones that were largest and had the highest survival under control conditions (271, 42E and 216) also had high fitness under elevated CO_2, whereas, under elevated O_3, clone 8L, a modest performer under control conditions, increased its growth and survival, whereas the other clones performed less well.

Discussion

Our results show that changing atmospheric composition can affect the genetic composition of forest tree populations. Genotype × environment interactions in aspen mortality shifted the expected clonal composition of forests under elevated CO_2 and elevated O_3, but not under the combination of these pollutants. These effects could largely be explained by differences in growth rate. Clones that exhibited high height growth under a given

Table 2 Fitted parameters for the best-fit model of *Populus tremuloides* survival (S8a), as well as the second and third best-fit models, S14a and S5a

		S8a	
Parameter		Estimate	95% CI
β_1	**Intercept**	**5.75**	**4.42, 7.06**
β_2	$+CO_2$	−0.56*	−1.94, 0.89
β_3	$+O_3$	−0.23	−1.71, 1.24
β_4	$+CO_2 + O_3$	−0.19	−1.71, 1.18
β_5	216	0.71*	−0.73, 2.17
β_6	**259**	−1.39*	−2.85, 0.08
β_7	**271**	**2.73**	**1.25, 4.37**
β_8	42E	−0.69*	−2.21, 0.76
β_9	8L	−0.40	−1.84, 1.13
β_{10}	$+CO_2 \times 216$	0.01	−1.62, 1.57
β_{11}	$+CO_2 \times 259$	−0.26	−1.86, 1.35
β_{12}	$+CO_2 \times 271$	−0.73*	−2.56, 0.88
β_{13}	$+CO_2 \times 42E$	1.38*	−0.31, 2.98
β_{14}	$+CO_2 \times 8L$	0.63*	−1.01, 2.20
β_{15}	$+O_3 \times 216$	0.73*	−0.99, 2.41
β_{16}	$+O_3 \times 259$	−0.66*	−2.26, 0.90
β_{17}	$+O_3 \times 271$	−0.13	−1.93, 1.76
β_{18}	$+O_3 \times 42E$	0.10	−1.62, 1.59
β_{19}	$+O_3 \times 8L$	1.18*	−0.48, 2.85
β_{20}	$+CO_2 + O_3 \times 216$	0.78*	−0.85, 2.54
β_{22}	$+CO_2 + O_3 \times 259$	0.02	−1.57, 1.61
β_{23}	$+CO_2 + O_3 \times 271$	−0.69*	−2.47, 1.11
β_{24}	$+CO_2 + O_3 \times 42E$	0.45	−1.14, 2.04
β_{25}	$+CO_2 + O_3 \times 8L$	0.13	−1.40, 1.69
α_2	**Ht inc**	**2.71**	**2.44, 3.03**
α_3	Age^2	−0.003	−0.01, 0.002
σ	**SE**	**9.47**	**7.86, 11.33**

Parameters whose 95% credible intervals do not overlap zero (that is, those with strong effects) are indicated in bold.
*Denotes those with moderately strong effects, where the credible interval extends at least twice as far on the positive side when the estimate is positive, or vice versa.

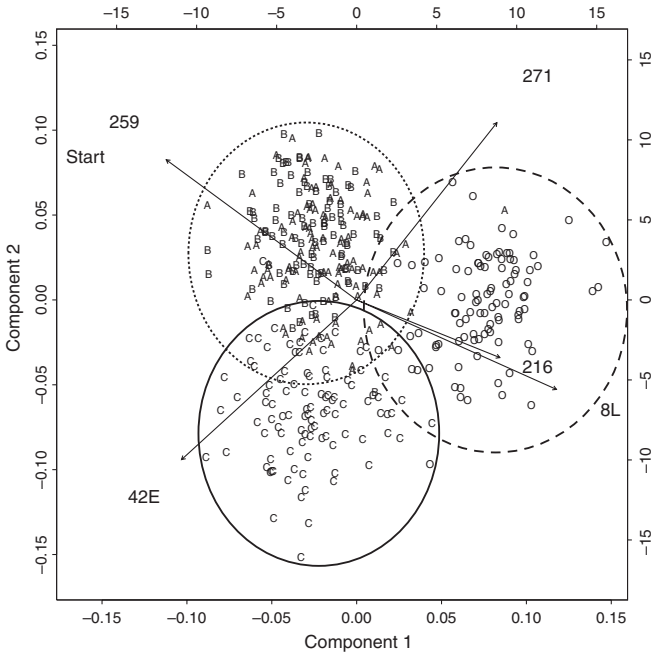

Fig. 3 Principal component analysis (PCA) diagram of simulated forest genotypic composition of *Populus tremuloides*. 'Start' indicates the initial condition of equal representation by all five clones. The other symbols represent the year-12 forest composition for 100 simulations: C, $+CO_2$ (enclosed by solid line); O, $+O_3$ (enclosed by dashed line); A, control conditions; B, $+CO_2 + O_3$ (both enclosed by dotted line).

Table 3 Average simulated *Populus tremuloides* clone rank abundance after 12 yr given equal initial numbers and best-fit Bayesian model

Control	$+CO_2$	$+O_3$	$+CO_2 + O_3$
271 (27.6%)	271 (27.2%)	271 (27.8%)	271 (25.8%)
216 (23.5%)	42E (23.3%)	216 (25.1%)	216 (25%)
42E (19.4%)	216 (22.2%)	8L (23.3%)	42E (20.8%)
8L (18.5%)	8L (19.6%)	42E (17.7%)	8L (18.7%)
259 (10.9%)	259 (7.7%)	259 (6.1%)	259 (9.8%)

Percentage abundance shown in parentheses.

treatment had higher survival and final relative abundances. Unlike the $+CO_2$ and $+O_3$ treatments, no clone × treatment growth effect was observed in the $+CO_2 + O_3$ treatment. We also showed that the observed differences in growth and mortality between clones could lead to longer term shifts in the average responses of forest trees when growth responses to CO_2 and O_3 are heritable. The expected response to selection also depends on the distribution of reproductive output.

Our growth and mortality results are consistent with previous studies of this site. Clone × treatment interactions in growth were observed in the $+O_3$ treatment just 3 yr into the Aspen FACE experiment (Isebrands *et al.*, 2001). Although an analysis based on the first 4 yr of Aspen FACE data concluded that aspen clone competitive rank did not change with treatment, that analysis used a measure based on the change in an individual's biomass relative to its neighbors (McDonald *et al.*, 2002) rather than survival vs mortality. Importance rank, a measure incorporating both survival and volumetric growth, had diverged by the seventh year in the elevated CO_2 and O_3 treatments (Kubiske *et al.*, 2007). Moreover, our results are consistent with previous studies showing that slow growth is associated with higher mortality in trees (Kobe & Coates, 1997; Wyckoff & Clark, 2002; Karnosky *et al.*, 2003; van Mantgem *et al.*, 2003).

Previous studies have found that elevated O_3 could lead to higher mortality in sensitive aspen clones (Berrang *et al.*, 1989; Karnosky *et al.*, 2003). However, although it is also known that there is genetic variation in CO_2 responses in aspen (Wang *et al.*, 2000; Isebrands *et al.*, 2001), it is not clear how increasing atmospheric CO_2 might affect population genetic composition. Moreover, the long-term effects of selection by pollutants on the growth responses of trees have not been considered. Our results suggest that aspen are likely to show a greater response to selection by O_3 than by elevated CO_2. This is consistent with many previous studies, which observed increases in plant O_3 tolerance following multigeneration exposure (Berrang *et al.*, 1989; Dickinson *et al.*, 1991; Heagle *et al.*, 1991; Whitfield *et al.*, 1997). By contrast, evolutionary responses to elevated CO_2 over

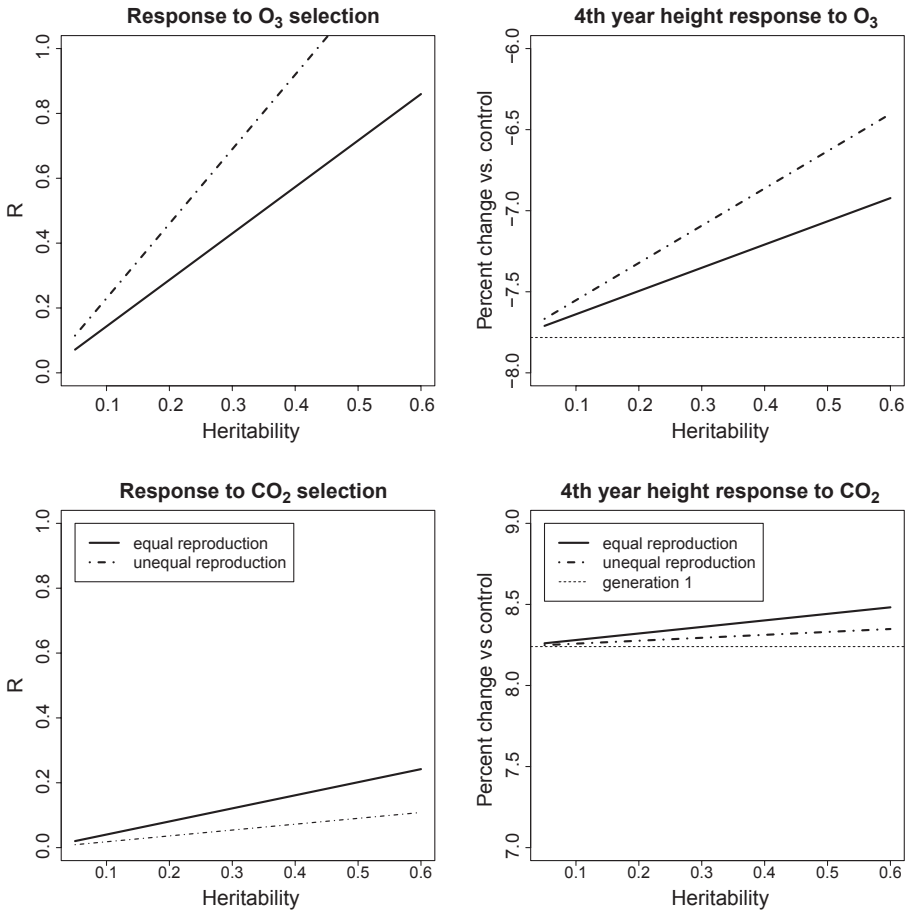

Fig. 4 Predicted response in *Populus tremuloides* to selection (*R*) by elevated O_3 and CO_2 (left panels) and the resulting change in fourth-year height growth response to these pollutants in the next generation (right panels) for a range of potential heritabilities. Responses assuming both equal reproductive success (solid line) and reproductive success proportional to diameter squared (dashed line) are shown. *R* = (response generation 2) – (response generation 1).

short timescales (less than six generations) tend to be weak (Ward *et al.*, 2000; Ziska & Bunce, 2000; Wieneke *et al.*, 2004; Steinger *et al.*, 2007), although Nakamura *et al.*'s (2011) study of adaptation in *Plantago asiatica* around a natural CO_2 spring suggests that significant evolution in growth and photosynthetic responses can occur. Moreover, a recent study by Cole *et al.* (2009) found variation in growth response to increasing ambient [CO_2] between wild aspen clones, indicating that there is phenotypic variation between genotypes on which selection could act.

However, in order to make better quantitative predictions of the response to selection, additional data are needed in three areas. First, what is the most relevant phenotypic trait? We chose the fourth-year height response for pragmatic reasons, but other studies have suggested that biomass, leaf area and O_3 uptake rate can be critical competitive traits (Haxeltine & Prentice, 1996; Hirose *et al.*, 1997; Poorter & Navas, 2003; Matyssek *et al.*, 2004; Kozovits *et al.*, 2005; Kubiske *et al.*, 2007). Second, what is the trait's heritability? Heritability depends on the amount of environmental variation (Gillespie, 2004), and therefore should be measured under the relevant field conditions. Finally, what is the distribution of reproductive success (Moran & Clark, 2012)? *Populus* trees at Aspen FACE were not reproductive when the experiment ended, so that data on the effect of clone and pollutant treatment on reproduction unfortunately do not exist. Previous studies have suggested that elevated CO_2 and/or O_3 can alter average age at maturity, seed and pollen production, and/or offspring performance in forest trees (LaDeau & Clark, 2006a,b; Darbah *et al.*, 2007), but little is known about variation in individual reproductive response to pollutants.

Interactions with other species could potentially alter both the initial pollutant response and response to selection. After 7 yr of growth, a competition index based on both survival and volumetric growth indicated that, relative to aspen (mostly clone 216), both birch and maple exhibited increased competiveness under elevated O_3, but decreased competitiveness under elevated CO_2 (Kubiske *et al.*, 2007). When competition is more important than the pollutant levels for growth and survival, this could limit selective responses (Wieneke *et al.*, 2004; Steinger *et al.*, 2007). Interactions with other environmental variables could also affect the outcome of selection. For instance, drought and heat stress can affect a plant's response to CO_2 and O_3, and vice versa (Fagnano & Merola, 2007; Bussotti, 2008; Uddling *et al.*, 2008; Darbah *et al.*, 2009). Therefore, predictions of selective responses to pollutants in the field must take into account concurrent changes in species interactions and other environmental variables.

When selection favors individuals with a strong CO_2 response or low O_3 sensitivity, this is likely to increase carbon fixation relative to nonevolving populations. Increases in O_3 tolerance, in particular, are likely to moderate the deleterious effects of this pollutant on plant growth. Clone 8L, which was responsible for much of the predicted decrease in height growth sensitivity in the

second generation, exhibited high individual trunk volume under elevated CO_2, indicating that height growth did not come at the expense of diameter growth (Table S6). Changes in the genotypic composition of plant populations could also have important effects on other community and ecosystem properties mediated by plant chemistry, including herbivore population dynamics (Lindroth *et al.*, 1993; Hwang & Lindroth, 1997; Mansfield *et al.*, 1999; McIntyre & Whitham, 2003; Osier & Lindroth, 2004), insect community composition (Bangert *et al.*, 2006; Bangert, 2008; Whitham *et al.*, 2008) and nutrient cycling (Madritch *et al.*, 2006; Whitham *et al.*, 2008).

In conclusion, we found that elevated CO_2 and O_3 can affect the clonal composition of an aspen population by changing the relative survival rates of aspen clones, that these changes in mortality are largely a result of clone × treatment interactions in growth rate, and that selection by elevated O_3 is likely to have a stronger effect on mean height growth responses in the next generation than is selection by elevated CO_2. If similar responses to selection by atmospheric pollutants, or other global change factors, occur in other dominant plant species, this could have profound effects on the genetic composition of populations and on competitive interactions between species, as well as on carbon sequestration and other ecosystem services.

Acknowledgements

Aspen FACE was principally supported by the US Department of the Environment (DOE) (grant no. DE-FG02-95ER62125) and the US Department of Agriculture (USDA) Forest Service, Northern Research Station. This work was conducted whilst E.V.M. was a Postdoctoral Fellow at the National Institute for Mathematical and Biological Synthesis, an Institute sponsored by the National Science Foundation, the US Department of Homeland Security and the USDA through NSF Award no. EF-0832858, with additional support from The University of Tennessee, Knoxville, TN, USA. We would also like to thank all reviewers and editors for their comments on the manuscript.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Difference between mean height in each treatment and mean height under control conditions for all clones.

Fig. S2 Mean annual height growth increment in meters for each aspen genotype in each treatment.

Fig. S3 Predicted and actual numbers of each genotype under each treatment, using posterior means for model S8a.

Table S1 Sample sizes of each aspen clone in each treatment

Table S2 Parameters included in tested mortality models

Table S3 Fit of all models, as measured by predictive loss

Table S4 Parameter estimates for second (S14a), third (S5a) and fourth (S7b) best-fit models

Table S5 Predicted mortality (best-fit model) of each clone under each treatment, with 95% credible intervals given equal initial numbers

Table S6 Mean individual trunk volume for each clone and treatment in year 12

Notes S1 Model description.

Notes S2 Justification of prior distributions, and sensitivity of results to changes in prior distributions.

Notes S3 Calculating selection differentials.

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