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**Intra-specific variation in juvenile tree growth under elevated CO<sub>2</sub> alone and with O<sub>3</sub> – A meta-analysis**

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**Running head:** GENETIC VARIATION IN GROWTH UNDER ELEVATED CO<sub>2</sub>

## Abstract

Atmospheric CO<sub>2</sub> concentrations are expected to increase throughout this century, potentially fostering tree growth. A wealth of studies have examined the variation in CO<sub>2</sub> responses across tree species but the extent of intra-specific variation in response to elevated CO<sub>2</sub> (eCO<sub>2</sub>) has, so far, been examined in individual studies and syntheses of published work are currently lacking. We conducted a meta-analysis on the effects of eCO<sub>2</sub> on tree growth (height, stem biomass and stem volume) and photosynthesis across genotypes to examine if there is genetic variation in growth responses to eCO<sub>2</sub> and understand their dependence on photosynthesis. We additionally examined the interaction between the responses to eCO<sub>2</sub> and O<sub>3</sub>, another global change agent. Most of the published studies so far have been conducted in juveniles and in *Populus* spp., although the patterns observed were not species-dependent. All but one study reported significant genetic variation in stem biomass, and the magnitude of intra-specific variation in the response to eCO<sub>2</sub> was similar in magnitude to previous analyses on inter-specific variation. Growth at eCO<sub>2</sub> was predictable from growth at ambient CO<sub>2</sub> ( $R^2 = 0.60$ ), and relative rankings of genotype performance were preserved across CO<sub>2</sub> levels, indicating no significant interaction between genotypic and environmental effects. The growth response to eCO<sub>2</sub> was not correlated with the response of photosynthesis ( $P > 0.1$ ) and, while we observed 57.7% average increases in leaf photosynthesis, stem biomass and volume increased by 36 and 38.5%, respectively, and height only increased by 9.5%, suggesting a predominant role for C allocation in ultimately driving the response to eCO<sub>2</sub>. Finally, best-performing genotypes under eCO<sub>2</sub> also responded better under eCO<sub>2</sub> & eO<sub>3</sub>. Further research needs include widening the study of intra-specific variation beyond the genus *Populus* and examining the interaction between eCO<sub>2</sub> and other environmental stressors. We conclude that significant potential to foster CO<sub>2</sub>-induced

44 productivity gains through tree breeding exists, that these programs could be based upon best-  
45 performing genotypes under ambient conditions, and that they would benefit from an increased  
46 understanding on the controls of allocation.

## Introduction

The concentration of atmospheric CO<sub>2</sub> has risen from 280 ppm before the industrial revolution to the current 400 ppm, and further increases are projected to occur during the current century (Blunden and Arndt 2014). CO<sub>2</sub> is a potent greenhouse gas and a major regulator of climate, and climate change may have positive or negative impacts on terrestrial ecosystems (IPCC 2014). However, elevated CO<sub>2</sub> *per se* is generally considered as either a positive or a negligible driver of terrestrial productivity (Norby et al. 2005; Palacio et al. 2014; Roderick et al. 2015). This is because Carbon is one of the (many) resources required for plant growth.

Higher C availability promotes tree growth directly through enhanced net photosynthesis and indirectly via water savings, at least in some ecosystems (Norby and Zak 2011). However, such promotion of tree growth can only be sustained when other resources necessary to sustain such growth enhancement (water, nutrients and radiation) are not co-limiting (Korner 2006; Tissue et al. 1993). After a transient increase in photosynthesis and growth, elevated CO<sub>2</sub> (eCO<sub>2</sub>) effects often dampen over time as a result of photosynthetic downregulation, starch accumulation, N dilution, or other processes (Norby et al. 2010; Tissue et al. 1993; Tissue et al. 1996). However, there are indications pointing towards a potential for long-term sustained eCO<sub>2</sub> effects via positive feedbacks to nutrient mineralization and uptake, which decrease nutrient limitations (Drake et al. 2011; Finzi et al. 2007; Peñuelas and Estiarte 1997). Despite all this complexity, eCO<sub>2</sub> benefits on productivity should be sustained over time provided trees are regularly watered and fertilized.

Increasing the productivity of forest plantations under a global change scenario is important for many reasons. Global population has constantly increased during the past century (up to the current 7.3 billion, United Nations Department of Economic and Social Affairs

Population Division 2015) and such increase is expected to continue (up to 9.7 billion by 2050, United Nations Department of Economic and Social Affairs Population Division 2015), leading to increased demands for wood and fiber. Recent studies indicate that we are approaching the point of requiring more phytomass than that available to sustain the human population (Schramski et al. 2015). Amongst the different alternatives to meet such enhanced demand, tree breeding offers the potential to increase productivity without the need to increase further the area currently dedicated to plantation forestry (Aspinwall et al. 2015).

Tree breeding relies, first, upon the existence of genetic variation in a given trait, such that genotypes with superior performance can be selected (Burdon 1977). It is also important to understand how the performance of genotypes varies with environment. Traditionally, a lack of genotype by environment ( $G \times E$ ) interaction has been considered as advantageous because, in its absence, the best-performing genotypes are always the same set of genotypes, regardless of the environment. Additionally, phenotypic plasticity has also been proposed to be beneficial, as a high positive response to an external driver such as elevated  $CO_2$  ( $eCO_2$ ) could increase productivity (Aspinwall et al. 2015).

There is a long-standing debate on whether the emphasis of tree breeding programs should be on producing genotypes suitable for specific environments or, on the contrary, on releasing genotypes simultaneously suited to a wide range of environments (wide vs. specific adaptation dilemma) (e.g. Basford and Cooper 1998; Sixto et al. 2014). It is thus important to clarify whether genotypes that increase productivity under both near-optimal and stressful conditions can be identified (Aspinwall et al. 2015). Identifying genotypes that are productive across a range of environments or management conditions is generally preferable, as it simplifies selection. This is particularly important under a global change scenario, where major

alterations to atmospheric chemistry and other processes will co-occur, such that responses to a single factor (e.g. eCO<sub>2</sub>) may not be the same as those when different factors are altered concomitantly (e.g. eCO<sub>2</sub> & ozone & drought, etc.).

Syntheses studies on how eCO<sub>2</sub> differentially affects inter-specific growth are abundant (Franks et al. 2013; Korner 2006; Wang et al. 2012), but a compilation of the published literature on intra-specific genetic variation in tree responses to eCO<sub>2</sub> has not yet been conducted. There are many aspects that remain to be clarified, such as the effects of eCO<sub>2</sub> on the “true trade-off” (sensu Grubb 2015) that is established between below- and above-ground allocation. Considering that increased C input increases the demand for other nutrients, we could expect increases in allocation of C to the roots to increase nutrient uptake (Ceulemans et al. 1999), such that stand productivity (in terms of aboveground biomass or wood production) remains equal under eCO<sub>2</sub>.

Here, we report the results of a meta-analysis on intra-specific responses of tree growth and gas exchange to elevated CO<sub>2</sub>. More specifically, we first sought to understand whether there was significant variation in response to eCO<sub>2</sub> across genotypes for these traits. Second, we aimed at understanding the relevance of G×E interactions by testing whether the best performing genotypes under eCO<sub>2</sub> can be predicted based on their performance under ambient CO<sub>2</sub> (aCO<sub>2</sub>). CO<sub>2</sub> manipulation experiments are expensive, and breeding programs would be more practical if they could be based upon aCO<sub>2</sub> responses. Third, we sought to shed more light on the mechanisms underlying genotypic differences in the response of trees to eCO<sub>2</sub>. We expect a higher photosynthesis than aboveground growth response to eCO<sub>2</sub>, indicating an important role for allocation. Finally, we aimed at understanding whether genotypes performing best under eCO<sub>2</sub> would also be least affected by the negative impacts of environmental stressors

(drought, warming, ozone [O<sub>3</sub>] concentrations). However, as we will describe in more detail, we could only assess the joint effect of CO<sub>2</sub> and O<sub>3</sub> on growth and photosynthesis. It has been well documented that exposing plants to O<sub>3</sub> often decreases photosynthesis and stomatal conductance (Bortier et al. 1999; Reich 1987) and O<sub>3</sub> has abruptly increased in the atmosphere in relation to pre-industrial levels.

## **Materials and Methods**

### *Literature survey and data collection*

We conducted extensive literature searches in Web of Science and Scopus with the expression “wood growth CO<sub>2</sub>” (March 2015) and we found 644 and 444 papers in each of these databases, respectively. We used such a general wording to minimize the number of papers that would be left out by applying a more stringent filter, therefore leading us to conduct a thorough examination of the current literature. As a consequence, most studies had to be discarded as they did not deal with intra-specific comparisons. After selecting all the papers dealing with intra-specific variation, we additionally searched all the references cited within those papers to find additional studies that could have gone unnoticed in our initial search.

We ended up with a total of 25 studies, from 14 independent facilities, which reported intra-specific variation across eight species (Table 1). These studies had been conducted using different types of facilities such as Free Air CO<sub>2</sub> Enrichment facilities (FACE), Open Top Chambers (OTC), polybags, greenhouses or growth chambers (Table 1). Intra-specific variation had been evaluated at the clonal level for most studies, although one study compared the performance across provenances (Spinnler et al. 2003). Because we were interested in intra-specific variability, we had to leave out a number of papers that assessed variability across



hybrid *Populus* clones (i.e. resulting from inter-specific crosses) (e.g. Radoglou and Jarvis 1990; Tognetti et al. 1999).

We did not apply any filter to the different possible growing conditions (water availability, fertilization, O<sub>3</sub>, etc.) as we originally intended to examine the interactive effects of eCO<sub>2</sub> under different environmental stressors. Unfortunately, we encountered a paucity of data for conditions other than non-limiting water and nutrients, and we could only assess CO<sub>2</sub> & O<sub>3</sub> joint effects (with nine studies available). The rest of studies had been conducted under ample water and nutrient supplies, with just a few exceptions examining CO<sub>2</sub> responses under different soil conditions, that were not enough for the meta-analysis (less than five cases).

We digitized all the data these papers contained (Plot Digitizer 2.6.6) for a total of 15 variables: height, stem biomass, stem volume, photosynthesis at growth CO<sub>2</sub>, diameter, biomass production, leaf area, leaf area index, root dry biomass, total dry biomass, wood density, leaf biomass, branch biomass, shoot-root dry biomass. However, different studies had been conducted for different purposes, and not all of these variables had always been measured. In fact, we concentrated our analysis only on height, stem biomass, stem volume and photosynthesis at growth CO<sub>2</sub> because this data was available for 8, 11, 6 and 12 studies respectively (five or less studies were available for the other variables).

### *Statistical analyses*

This meta-analysis was conducted following the approach of Wang et al. (2012), with only a few exceptions as noted below. When a time series was reported, we only used the last value per treatment and study for the growth variables, as that ensured plants were at the oldest stage possible. However, because photosynthesis (which was often reported at light saturation) is

highly variable, we used mean seasonal values whenever possible. Plant age ranged from 60 days to seven years, depending on the study. We performed unweighted analysis using the log response ratio (RR) to quantify the response to eCO<sub>2</sub> because this is common practice in ecological meta-analyses (Curtis and Wang 1998; Hedges et al. 1999; Wang et al. 2012). Confidence intervals (CIs) for effect-size estimates were calculated by bootstrapping the unweighted data with a resampling of 1,000 iterations. We considered eCO<sub>2</sub> had a significant effect when the interval captured by the CI did not overlap zero.

In order to avoid publication bias, we calculated Rosenthal's fail-safe number (or the number of non-published studies with non-significant results necessary to affect our conclusions) (Rosenthal 1979) using the library *metafor* (Viechtbauer 2010). We did not observe significant publication bias for any of the results presented. Further details on the meta-analysis procedure can be found in Wang et al. (2012). Although meta-analysis calculations were performed on log RR, we report the results as % change, for simplicity.

After quantifying eCO<sub>2</sub> responses and associated CIs, we assessed whether the relative genotype performance at aCO<sub>2</sub> was maintained at eCO<sub>2</sub> by regressing the values at eCO<sub>2</sub> against those at aCO<sub>2</sub> and also by examining rank changes. Because the studies had been performed on plants from different species and ages, they showed contrasting differences in the four traits measured in this study. Thus, prior to analyses, we conducted a normalization by dividing the value for a given trait, clone and CO<sub>2</sub> concentration by the maximum value for that trait within that study (that is, a linear rescaling assuming the minimum is zero), so that values for all studies would be within the same axis of variation and, therefore, comparable. To examine rank changes, all genotypes within one study were given a number based on how they ranked in terms of performance (1<sup>st</sup>, 2<sup>nd</sup> ...), and regressed the ranking at eCO<sub>2</sub> against the ranking at aCO<sub>2</sub>.

All these regressions were first computed separately for each species and when the number of clones was higher than two. We then conducted a standardized major axis estimation (Warton et al. 2012) to test for significant differences in slope and intercept across species between  $eCO_2$  vs.  $aCO_2$  normalized values and rankings and, when those were not significant, we conducted a single mixed model analysis (Bates et al. 2014; Fox and Weisberg 2011; Nakagawa and Schielzeth 2014) for all species that included values at  $aCO_2$  as continuous explanatory variables and species and facility as random variables.

To understand the role of C input in driving growth responses, we regressed the response to  $eCO_2$  in height, stem biomass and stem volume against the response in photosynthesis separately for each species. To understand the interaction between  $CO_2$  and  $O_3$ , we regressed normalized values at  $eO_3$  and, also at  $eCO_2$  &  $eO_3$ , against normalized values at ambient  $CO_2$  and  $O_3$  levels. After testing whether the response across genotypes varied as a function of species with standard major axis estimation, we conducted mixed model analyses following the same logic as that explained in the previous paragraph. All analyses were performed in the R software environment (R Core Team 2015).

## Results

### *Intra-specific variation in the response to $eCO_2$ in growth and photosynthesis*

We observed that tree growth and photosynthesis significantly increased in response to  $eCO_2$  across studies (Fig. 1). However, there were strong differences in the response to  $eCO_2$  across traits. For instance, photosynthesis showed a much larger mean  $eCO_2$  response (57.7%) than height (9.5%). The response of stem biomass (36%) was similar to that of stem volume (38.5%) and, overall, the error bars (95% CI) between these two variables and photosynthesis (in log

response ratio, but also in percent response) crossed (Fig. 1). However, the responses of stem volume and stem biomass were almost four times higher than in height.

When examining individual studies, we observed that the response to eCO<sub>2</sub> was almost always significant, as the 95% CI (in log response ratio, but also in percent response) only crossed the 0 response line in one study for height, stem biomass and photosynthesis (Fig. 2). The trait that showed the highest response was photosynthesis (up to 176% increase, Fig. 2d), while the trait with the lowest response was height (Fig. 2a). Stem biomass and stem volume showed intermediate responses (Fig. 2b, c).

We observed a wide range of variability in response to eCO<sub>2</sub> across genotypes in the different studies, as indicated by the large CIs in Fig. 2. To compare the magnitude of intra- and inter-specific variability, we visually compared the 95% CI in our analysis with those reported by Wang et al. (2012), who assessed inter-specific variability across a large number of species, including those of the present study. Overall, we observed that, for those traits also reported by Wang et al. (i.e. stem biomass and photosynthesis), the extent of intra-specific variation was, at least, as large as that of inter-specific variation according to the length of CI bars in Fig. 2. For instance, the mean length of the error bars in stem biomass within a study was 30% (Fig. 2), and the response in Wang et al. (2012) varied between by 16% (21% - 37%). It is important to note, however, that the comparison with Wang et al. (2012) is merely indicative. The CI could be lower in Wang et al. (2012) simply because of the much larger sample size used in that study. Nonetheless, our results indicate a significant degree of intra-specific variability.

*Genotype × environment interaction in response to eCO<sub>2</sub>*

Our results showed a strong positive association between values at elevated and at ambient CO<sub>2</sub> for all traits except photosynthesis (Fig. 3). After fitting species-specific regressions (for those species represented by three or more genotypes), the standardized major axis analysis revealed that there were no significant differences in the slope or intercept across species. We thus conducted a mixed model analysis (as described in methods) and observed that the R<sup>2</sup> of the regression between normalized height, stem biomass and volume at eCO<sub>2</sub> vs. normalized values measured at aCO<sub>2</sub> were 0.83, 0.60 and 0.80, respectively (Fig. 3). However, photosynthesis at eCO<sub>2</sub> was not related to that at aCO<sub>2</sub> (at  $P < 0.05$ ).

There was a significant and positive relation between the genotype rankings at aCO<sub>2</sub> and at eCO<sub>2</sub> for height, stem biomass and stem volume within a species (Fig. 4). That is, a particular genotype tended to maintain its level of performance after exposure to eCO<sub>2</sub>, relative to the performance of the other genotypes. As with normalized values, standard major axis regression performed on rankings indicated no significant differences across species, and the mixed model analysis revealed that 65%, 74% and 87% of the variability in the ranking at eCO<sub>2</sub> could be predicted from that at aCO<sub>2</sub> for height, stem biomass and stem volume, respectively (Fig. 4). However, the genotype rankings for photosynthesis at aCO<sub>2</sub> were not predictive of genotype rankings at eCO<sub>2</sub> ( $P > 0.05$ ). It is important to note that the analysis of genotypic rankings only considered the mean (and not the error) value for a genotype. That is, the trait value for a genotype ranked first will be higher than for a genotype ranked second, but the difference in the actual value may not be statistically significant.

### *Photosynthesis as a driver of growth*

Photosynthesis always showed a significantly higher response to eCO<sub>2</sub> than height, and tended towards a higher response than stem biomass and stem volume (Figs. 1, 2). However, the response of the growth traits could not be predicted from the response of photosynthesis. The response of stem biomass to eCO<sub>2</sub> was only significantly correlated with the response of photosynthesis in one species (*Picea sitchensis*,  $P = 0.02$ ,  $R^2 = 0.95$ ), but the slope of the response was negative (-0.56), indicating that leaf level photosynthesis alone did not explain the genotypic differences in height increase in response to eCO<sub>2</sub> (Fig. 5a). Similarly, the response of height was uncorrelated with that of photosynthesis for all species ( $P > 0.05$ ; Fig. 5b). Given the lack of significant relationships at the species level, we did not attempt to compare differences in slope or intercept of the regression across species. We were unable to correlate photosynthesis with stem volume due to lack of available studies (not all studies that measured photosynthesis also measured all growth traits).

### *Joint effects of elevated CO<sub>2</sub> and ozone on genotype growth and photosynthesis*

We generally, but not always, observed reductions in height, stem volume and photosynthesis after exposing genotypes to elevated ozone concentrations (eO<sub>3</sub>), as indicated by values under the 1:1 line in Fig. 6 a-c (there were not enough data to assess effects on stem biomass). However, genotypes growing under both eCO<sub>2</sub> & eO<sub>3</sub> generally achieved similar values in height, stem volume and photosynthesis as genotypes growing under ambient conditions (Fig. 6). Additionally, we observed that the normalized response of genotype height, volume and photosynthesis to both, eO<sub>3</sub> alone, and to eCO<sub>2</sub> & eO<sub>3</sub>, was correlated ( $P > 0.001$ ,  $0.31 < R^2 < 0.95$ ).

0.86) with the values at ambient conditions (Fig. 6, except for photosynthesis under elevated eCO<sub>2</sub> & eO<sub>3</sub>).

## **Discussion**

### *Genotype variation in response to elevated CO<sub>2</sub>*

Elevated CO<sub>2</sub> had a significant positive effect on tree growth and gas exchange, but the magnitude of the response differed among species and genotypes. Overall, the increase in stem biomass was similar in magnitude to that commonly reported on studies examining inter-specific variation (Ainsworth and Rogers 2007; Wang et al. 2012). However, we observed an increase in photosynthesis (57.7%) much larger than that typically documented in Wang's meta-analysis (mean of 19%, Fig. 2). This large response was partially driven by a single study (#13 in Table 1) reporting an increase of 176%; if this study is not taken into account, the photosynthesis response becomes a 46.9% increase (still higher than the result reported in Wang et al. 2012). The higher response in photosynthesis in the present study could be partly explained by the dominance of more-responsive juvenile trees in this study (mostly between six months to three years), as has been previously documented (Kostiainen et al. 2014; Wang et al. 2012). This large photosynthetic response to eCO<sub>2</sub> could also be biased by the relatively low number of published studies on intra-specific variation relative to those on inter-specific variation, which overall limits the degree of generalization from these results. However, given that we observed a large degree of genetic variation in growth and its response to eCO<sub>2</sub>, we can conclude that there is potential for selecting eCO<sub>2</sub>-responsive genotypes in tree breeding programs.

*Lack of interactions between genotype and environment in response to eCO<sub>2</sub>*

We did not find conclusive evidence for large G×E interactions affecting growth traits in response to eCO<sub>2</sub>. This is because height, stem biomass and stem volume at eCO<sub>2</sub> could be reasonably well predicted from the values at aCO<sub>2</sub> (Fig. 3) and, additionally, because the relative genotype rankings for growth at aCO<sub>2</sub> were maintained also at eCO<sub>2</sub> (Fig. 4). If the response to eCO<sub>2</sub> had depended on genotype, then we would have expected that values at the “new” environment (eCO<sub>2</sub>) would not be predictable from those at the “old” environment (aCO<sub>2</sub>), and also the ranking of the genotypes should not have been preserved across environments. Overall, these results indicate that selecting best performers under current conditions should also lead to selecting the best performers under eCO<sub>2</sub>.

The lack of relationship in photosynthesis at eCO<sub>2</sub> and that at aCO<sub>2</sub> at the intra-specific level is intriguing and deserves further exploration. Following the same logic as in the previous paragraph, it would indicate that there was a significant interaction between CO<sub>2</sub> levels and genotypes. However we have to consider additional possibilities. For instance, unlike growth, which is the result of the accumulated life history of the plant, instantaneous gas exchange measurements strongly fluctuate over short time periods, and that might have influenced the response obtained in the different studies.

*Carbon allocation, and not photosynthesis, as major driver of growth responses*

Overall, the most responsive variable to eCO<sub>2</sub> was photosynthesis. However, the intra-specific relationships between photosynthesis and growth were irrelevant. First of all, the response of photosynthesis was much larger than that of growth traits (specially height). It is important to note that photosynthesis is reported here at the leaf, and not at the whole-tree, level. It is likely



that trees growing under eCO<sub>2</sub> had higher leaf area than trees growing at aCO<sub>2</sub>, such that the response of total plant photosynthesis to eCO<sub>2</sub> would have been much larger than the leaf response reported here. It thus seems like a mismatch between C input and aboveground growth occurred, as the former seemed to be much larger than the latter. Indeed, similar to other studies dealing with inter-specific associations (Fatichi et al. 2014; Körner 2009), intra-specific relationships between photosynthesis and growth responses to eCO<sub>2</sub> were not significant.

We did not have enough data to fully examine responses of stem diameter but, overall, our results suggest that the positive growth responses were driven by a coordinated change in stem diameter and in stem height. We observed height and volume to increase, respectively, by 9.5% and 38.5%. Stem volume is often approximated by the volume of a cylinder ( $\pi$  times radius squared times height) and, if diameter had also increased by ~10%, then we would expect stem volume to have increased roughly by 33%, which is similar (although slightly lower) than the observed 38.5%.

The finding that increases in C uptake were larger than in growth and that growth could not be predicted from photosynthesis indicate that, while eCO<sub>2</sub> has a positive effect on photosynthesis, it may not necessarily translate into a linear increase in height or in stem biomass. In fact, our results point towards increases in allocation of C to other C sinks such as belowground growth, respiration, exudation or defenses, to name a few, as important responses to eCO<sub>2</sub> (Körner 2003). Plants are co-limited by many resources and, as limitation for one resource relaxes, the limitation for another resource increases, with important feedbacks for C allocation. For instance the increase of a belowground resource, such as N, usually increases allocation towards aboveground growth (Franklin et al. 2012; Grubb 2015). On the contrary, a large availability of an aboveground resource such as CO<sub>2</sub> could have stimulated an increase in

the allocation of C belowground, to increase nutrient uptake and sustain the increased demands for growth derived from the increase in one resource (Ceulemans et al. 1999; Franklin et al. 2012). Another possible fate for the extra C inputs under eCO<sub>2</sub> could be additional investments in defense structures. For instance, Cseke et al. (2009) observed increases in active defense compounds in response to eCO<sub>2</sub> for the most responsive *P. tremuloides* genotypes, and increasing cell wall thickness and passive defense compounds (lignin, phenypropanoid) under moderate responses to eCO<sub>2</sub>.

A long-term question in eCO<sub>2</sub> research has been whether or not photosynthesis experiences down-regulation (Tissue and Oechel 1987; Warren et al. 2014). The apparent lack of down-regulation (as indicated by higher photosynthesis at growth CO<sub>2</sub> in plants grown at eCO<sub>2</sub> relative to plants growing at aCO<sub>2</sub>) may reflect tree response to non-limiting well-watered and fertilized conditions relevant for intensively managed forestry plantations, and data from primarily juvenile, fast-growing trees after relatively short (<10 years) exposures to eCO<sub>2</sub>. However, our data adds to an increasing body of evidence which suggests that understanding the fate of C after assimilation and its allocation to different plant organs and/or defense strategies could be more important for optimizing selection responses to eCO<sub>2</sub> than understanding C assimilation *per se* (Fatichi et al. 2014).

#### *Tree growth responses to the combined effects of ozone and eCO<sub>2</sub>*

It has been well documented that exposing plants to ozone often decreases photosynthesis and stomatal conductance (Bortier et al. 1999; Reich 1987). Consistent with previous works, height, stem volume and photosynthesis all showed a generally negative response to eO<sub>3</sub>. This effect is often explained in terms of damaging chloroplasts, reducing Rubisco content, reducing stomatal

conductance or interfering with the flow of C to the roots in chlorophyll concentration (Kostiainen et al. 2014; Krupa et al. 2000). However, eCO<sub>2</sub> might overcome the negative effect of eO<sub>3</sub>. Indeed, genotypes grown under eCO<sub>2</sub> & eO<sub>3</sub> showed a similar response to those grown under ambient conditions. Moreover, there was generally a significant and positive intra-specific association between values at eO<sub>3</sub> and at eCO<sub>2</sub> & eO<sub>3</sub> with values under ambient environmental conditions, further indicating a lack of G×E interactions and that by selecting for best-performing genotypes under ambient conditions, we are also selecting for the genotypes that will show superior performance under eO<sub>3</sub> and under eCO<sub>2</sub> & eO<sub>3</sub>.

#### *Current knowledge, gaps and future research directions*

Overall, we can conclude that: 1) significant potential to foster CO<sub>2</sub>-induced productivity gains through tree breeding exists; 2) genotype growth under eCO<sub>2</sub> is highly predictable based on genotype growth at aCO<sub>2</sub>; 3) large increases in photosynthesis at the genotype level do not proportionally translate into aboveground growth increases of comparable magnitude, where C allocation to belowground growth or to other C sinks may be the main pools of the additional C uptake; and 4) eCO<sub>2</sub> at least partially overcomes some of the negative effects of O<sub>3</sub> on tree growth, with a good correspondence among genotypic means when comparing values at ambient with values at both, eO<sub>3</sub> and eCO<sub>2</sub> & eO<sub>3</sub>. The application of these conclusions to field settings, however, comes with a number of caveats sprouting from the conditions upon which the experiments were performed. Indeed, a major conclusion of this study is that important gaps and biases still exist on our current knowledge of intra-specific responses to tree growth to eCO<sub>2</sub>.

Most of the studies analyzed here were conducted in *Populus* (16 out of the 25 studies), and about a fourth of all the papers analyzed came from a single facility (AspenFACE, Table 1) so further research will be needed to fully understand the existing genetic variation in response to eCO<sub>2</sub> across a broader range of species. We also encountered a large bias towards juvenile trees, and future studies could focus on mature forest ecosystems, to the extent that this is possible. We have noted the importance of allocation in driving the eCO<sub>2</sub> growth response; however, due to the expensive nature of eCO<sub>2</sub> treatments, most of the studies here examined were performed under non-competitive conditions (i.e. in the absence of competition for light interception). Understanding how changes in allocation in response to eCO<sub>2</sub> are affected by aboveground competition should be at the forefront of our research efforts, as limiting light could increase C investment into primary growth. On a related note, the observation that stem volume responses were similar to those of stem biomass suggests that wood density was not affected by eCO<sub>2</sub>. Although this result is only tentative, it indicates that future research needs include the assessment of genetic variability in wood quality under eCO<sub>2</sub>.

We note that our results were conducted under non limiting conditions of nutrients like N and P. Considering that belowground allocation increases under limited soil fertility, increases in CO<sub>2</sub> under limited nutrients could exacerbate this response. Similarly, given the multifactorial nature of global change, we need more studies that concomitantly address the response to CO<sub>2</sub> and other climate change agents, such as temperature and water stressors. Similarly, understanding the effects of biotic agents, such as pests and pathogens, on the response to eCO<sub>2</sub> is also an important knowledge gap (Näsholm et al. 2014).

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## Figure legends

**Figure 1.** Responses to eCO<sub>2</sub> of height, stem biomass, stem volume and photosynthesis. Each data point represents the mean ( $\pm$  95% CI) from all papers for each variable (Table 1). Significant responses occur when the error bars do not cross the 0% line (at  $P < 0.05$ ).

**Figure 2.** Intra-specific variation in the response to eCO<sub>2</sub> for (a) height, (b) stem biomass, (c) stem volume and (d) photosynthesis for each study (see Table 1 for further details for each study). The vertical dotted line separates data for different species and species names are indicated by Bp (*Betula pendula*), Ec (*eucalyptus camaldulensis*), Fs (*Fagus sylvatica*), Hb (*Hevea brasiliensis*), Pa (*Picea abies*), Ps (*Picea sitchensis*), Pd (*Populus deltoides*) and Pt (*Populus tremuloides*). The range in intra-specific variability is indicated by the mean and the 95% CI bars. To compare the range in intra-specific variability with the range in inter-specific variability, we reproduce the results from a recent meta-analysis on inter-specific responses to eCO<sub>2</sub> when available (Wang et al. 2012). The dashed horizontal line indicates no response to eCO<sub>2</sub>.

**Figure 3.** Comparison of genotype values at elevated vs. ambient CO<sub>2</sub> for normalized (a) height, (b) stem biomass, (c) stem volume and (d) photosynthesis. Each data point represents the value of a given genotype in a given study. The dashed line indicates the 1:1 relationship. The different continuous lines indicate the result of species-specific least squares fitting (with non-significant relationships indicated by a lack of best-fit line for that species). When no significant differences in the slope across species were present, a single model was fitted to all the data, and

its goodness of fit is indicated by the  $p$ - and  $R^2$ - values at the bottom of each panel. Different colors indicate different species (with abbreviations as in Fig. 2).

**Figure 4.** Comparison of genotype rankings at elevated vs. ambient CO<sub>2</sub> for (a) height, (b) stem biomass, (c) stem volume and (d) photosynthesis. Each data point represents the ranking of a given genotype in a given study. The dashed line indicates the 1:1 relationship. The different continuous lines indicate the result of species-specific least squares fitting (with non-significant relationships indicated by a lack of best-fit line for that species). When no significant differences in the slope across species were present, a single model was fitted to all the data, and its goodness of fit is indicated by the  $p$ - and  $R^2$ - values. Different colors indicate different species (with abbreviations as in Fig. 2). Overlapping values may be hidden.

**Figure 5.** Comparison of the response to eCO<sub>2</sub> in stem biomass (a) and height (b) against that in photosynthesis. Data points represent the percent change response for a clone with joint reports of photosynthesis and biomass (a) or photosynthesis and height (b). Relationships between photosynthesis and stem volume could not be examined due to a lack of data. The different continuous lines indicate the result of species-specific least squares fitting (with non-significant relationships indicated by a lack of best-fit line for that species). Different colors indicate different species (with abbreviations as in Fig. 2).

**Figure 6.** Comparison of genotype means for plants growing under elevated O<sub>3</sub> vs. plants growing under ambient CO<sub>2</sub> (a-c); and for plants growing under elevated O<sub>3</sub> & elevated CO<sub>2</sub> vs. plants under ambient CO<sub>2</sub> (d-f). Each data point represents the value of a given genotype in a

657 given study. The dashed line indicates the 1:1 relationship. “photos” stands for photosynthesis.  
658 The different continuous lines indicate the result of species-specific least squares fitting (with  
659 non-significant relationships indicated by a lack of best-fit line for that species). When no  
660 significant differences in the slope across species were present, a single model was fitted to all  
661 the data, and its goodness of fit is indicated by the  $p$ - and  $R^2$ - values at the bottom of each panel.  
662 Different colors indicate different species (with abbreviations as in Fig. 2).  
663  
664

**Table 1.** Account of genotypic variation among genotypes of different species in response to elevated CO<sub>2</sub> for height, stem biomass, stem volume and photosynthesis. Letters indicate whether (“Y” =yes) or no (“N”) significant genotypic effects were reported in the original study, (“NR” indicates genotypic effects were not reported) and blanks indicate no data was available. Some references may provide results for the same facility, but provide data for different variables or clones. OTC and FACE indicate Open Top Chamber and Free Air CO<sub>2</sub> Enrichment, respectively.

| References                  | Study number | Species                         | Experimental facility | # Genotypes | Height | Stem biomass | Stem volume | Photosynthesis |
|-----------------------------|--------------|---------------------------------|-----------------------|-------------|--------|--------------|-------------|----------------|
| Riikonen et al. (2004)      | 1            | <i>Betula pendula</i>           | OTC                   | 2           |        | N            | N           |                |
| Riikonen et al. (2005)      | 2            | <i>Betula pendula</i>           | OTC                   | 2           |        |              |             | Y              |
| Resco de Dios et al. (2016) | 3            | <i>Eucalyptus camaldulensis</i> | Greenhouse            | 6           |        | Y            |             | Y              |
| Spinnler et al. (2003)      | 4            | <i>Fagus sylvatica</i>          | OTC                   | 4           |        | Y            |             |                |
| Devakumar et al. (1998)     | 5            | <i>Hevea brasiliensis</i>       | Polybags              | 2           | NR     | NR           |             |                |
| Spinnler et al. (2003)      | 6            | <i>Picea abies</i>              | OTC                   | 8           |        | Y            |             |                |
| Townend (1993)              | 7            | <i>Picea sitchensis</i>         | Growth Chambers       | 4           |        | Y            |             |                |
| Centritto et al. (1999)     | 8            | <i>Picea sitchensis</i>         | OTC                   | 4           | NR     | Y            |             |                |
| Centritto and Jarvis (1999) | 9            | <i>Picea sitchensis</i>         | OTC                   | 4           |        |              |             | NR             |
| Ceulemans et al. (1995)     | 10           | <i>Populus deltoides</i>        | OTC                   | 2           |        |              |             | NR             |
| Ceulemans et al. (1996)     | 11           | <i>Populus deltoides</i>        | OTC                   | 2           | Y      | Y            | Y           |                |
| Ceulemans et al. (1997)     | 12           | <i>Populus deltoides</i>        | Greenhouse            | 2           |        |              |             | NR             |
| Ceulemans et al. (1997)     | 13           | <i>Populus deltoides</i>        | OTC                   | 2           |        |              |             | NR             |
| Tupker et al. (2003)        | 14           | <i>Populus deltoides</i>        | Greenhouse            | 5           |        | Y            |             |                |
| Dickson et al. (1998)       | 15           | <i>Populus deltoides</i>        | OTCs                  | 4           | Y      | Y            |             |                |
| Isebrands et al. (2001)     | 16           | <i>Populus tremuloides</i>      | FACE                  | 5           | Y      |              | Y           |                |
| Noormets et al. (2001)      | 17           | <i>Populus tremuloides</i>      | FACE                  | 2           | Y      |              | Y           | Y              |
| Kubiske et al. (2007)       | 18           | <i>Populus tremuloides</i>      | FACE                  | 5           | N      |              | Y           |                |
| Cseke et al. (2009)         | 19           | <i>Populus tremuloides</i>      | FACE                  | 2           |        |              | Y           | N              |
| Noormets et al. (2010)      | 20           | <i>Populus tremuloides</i>      | FACE                  | 2           |        |              |             | Y              |
| Tupker et al. (2003)        | 21           | <i>Populus tremuloides</i>      | Greenhouse            | 2           | N      |              |             |                |
| Darbah et al. (2010)        | 22           | <i>Populus tremuloides</i>      | FACE                  | 2           |        |              |             | NR             |
| Wang et al. (2000)          | 23           | <i>Populus tremuloides</i>      | OTC                   | 6           |        | Y            |             | Y              |
| Dickson (2001)              | 24           | <i>Populus tremuloides</i>      | OTC                   | 2           |        |              | NR          |                |
| Kubiske et al. (1998)       | 25           | <i>Populus tremuloides</i>      | OTC                   | 2           |        |              |             | Y              |













