



Forests for the future: A Meta-analysis on genetic variation in wood production under elevated CO₂



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Abstract

There is some uncertainty on the climate of the 21st century, but increases in CO₂ concentrations are certain. When plant growth is carbon limited, increasing CO₂ concentration should foster tree growth. A wealth of studies have examined the variation in CO₂ responses across tree species but understanding the drivers of intra-specific variation has, so far, been conducted in individual studies and we are still lacking a synthesis of published work. I conducted a meta-analysis on the effects of elevated CO₂ (eCO₂) on tree growth (height, stem biomass and stem volume) and photosynthesis across genotypes to understand if there was genetic variation in responses to eCO₂ and examine the underlying mechanisms, and to additionally understand the interactions between eCO₂ and other global change agents, like O₃. I observed that most of the published studies so far have been conducted in *Populus* and that the range of variation in eCO₂ responses across genotypes (from 11% to 42% for stem biomass) was similar to previous analyses on inter-specific variation (21%-37% for stem biomass, (Centritto et al. 1999, DeLucia et al. 2005), indicating a large potential for tree breeding. Growth at eCO₂ was highly predictable from the growth at ambient CO₂ (aCO₂, $R^2 = 0.83$), and relative rankings of genotype performance were preserved across CO₂ levels, indicating no significant interaction between genotypic and environmental effects. The growth response to eCO₂ was highly correlated with the response of photosynthesis ($R^2 = 0.83$) but, while we observed 106% increases in photosynthesis, height only increased by 11%, indicating a predominant role for C allocation in ultimately driving the response to eCO₂, where allocation to primary growth may not be a primary objective of increased assimilation. I additionally observed that best-performing genotypes

under eCO₂ also respond better under eCO₂ x O₃ although not equally. More over, the effect of O₃ on tree growth was observed and I found negative percent responses. Further research needs on this area include the study of intra-specific variation outside the genus *Populus* and on the interaction between eCO₂ and other environmental stressors. Overall, my results indicate significant potential for tree breeding programs to foster CO₂ induced productivity gains, where lack of genotype by environment interactions simplify selection.

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Contents

| | |
|-----------------------------------------------------------------------------------------------------------------------|-----|
| Abstract | i |
| Acknowledgement | iii |
| Introduction | 1 |
| Materials and Methods | 4 |
| Literature survey and data collection | 4 |
| Meta-analysis | 5 |
| Results | 7 |
| Intra-specific variation in the response to elevated CO ₂ growth and photosynthesis across genotypes | 7 |
| Genotype x environment interaction in response to eCO ₂ | 12 |
| Photosynthesis as a driver the growth response | 16 |
| Effects of CO ₂ x O ₃ on genotype growth and photosynthesis | 17 |
| Discussion | 20 |
| Genotype variation in response to elevated CO ₂ | 20 |
| Lack of interactions between genotype and environment in response to eCO ₂ | 21 |
| Carbon allocation, and not photsynthesis, was the major driver of growth responses | 21 |
| Tree growth response to ozone and ozone plus CO ₂ concentration - | 22 |
| Conclusion | 24 |
| References | 25 |

List of Tables

| | |
|---------------------------------------------------------|----|
| 1. Summary of genotypic variation among clones of | 11 |
|---------------------------------------------------------|----|

List of Figures

| | |
|-----------------------------------------------------------------------|----|
| 1. Response to eCO ₂ of stem biomass..... | 7 |
| 2. Intra-specific variation in the response to eCO ₂ | 9 |
| 3. Comparison of genotype values at elevated | 13 |
| 4. Comparison of genotypes grown in ambient CO ₂ | 15 |
| 5. Comparison of the response to eCO ₂ | 16 |
| 6. Comparisons of the % change for variables | 18 |
| 7. Comparisons of the % change for variables | 19 |

Introduction

One of the major global problems today is the rapid increase of atmospheric CO₂ and its influence on global climate change. The global carbon balance has changed substantially with the increase of anthropogenic emissions resulting in climate warming (Raupach et al. 2007).

Forests play a significant role in the climate system as large carbon reservoirs. In fact, the balance between photosynthesis and respiration is currently thought to assimilate 25-30% of fossil fuel emissions (Hole 2006, Canadell 2010), with major C sinks currently in middle and high latitudes of the northern hemisphere (Fang et al. 2014). Terrestrial C sequestration is the result of the immobilization of C through growth, and understanding the evolution of the terrestrial C sink under climate change is the topic of much debate (Körner 2006).

One of the most important drivers of forest growth under climate change is CO₂. When trees are exposed to elevated CO₂ concentrations (eCO₂) photosynthesis rates increase in the short-term (Al, R.Ceulemans et al. 1994). In the longer term, a downregulation of photosynthesis is often observed, specially under nutrient limitations (Tissue et al. 1997). However, when trees are grown under ample nutrient supply, as may be the case of fertilized forestry plantations, then we can expect sustained increases in growth over time (Oren et al. 2001). Under eCO₂, trees additionally decrease their rate of transpiration and of stomatal conductance, therefore increasing water use efficiency (Dickson et al. 2001, Krishi et al. 1998), (but not always, cf. Bauweraerts et al. 2014). This may consequently lead to improved water balance and permit tree canopies to retain greater leaf area (Kostiainen et al. 2014), further fostering increased tree growth.

How this increase in C input under eCO₂, in the absence of nutrient limitations, relates to increases in growth is the topic of much discussion (Esser et al. 2011), with recent work pointing towards increases in C allocation belowground as predominant under eCO₂. For example, the number of roots and root length have been documented to increase substantially under elevated CO₂ (Reich et al. 2006, Thornton et al. 2007, Esser et al. 2011). However, the positive effects of eCO₂ on growth could be counter-balanced by other global change drivers, such as water scarcity, increased warming and ozone concentrations (O₃), to name a few (Riikonen et al. 2005). For example, ozone often decreases photosynthesis and stomatal conductance by affecting carbon fixation, light usage efficiency and amount and activity of Rubisco (Noormets et al. 2001, Nunn et al. 2005, Riikonen et al. 2005), subsequently resulting in growth reductions. An important research question is thus to understand whether the positive effects of eCO₂ will be maintained, at least to some degree, under the negative impacts of increasing atmospheric stressors.

Increases in global population raise an additional challenge to forestry, as population increases unavoidably lead to decreases in forest area. According to the United Nation's World Population Prospect Report, the global population is currently 7.3 billion, and it is expected raise up to 9 billion by the year 2050 (United Nations 2013). Forest plantations have been established worldwide to satisfy the demands of such an increasing population. While forest plantations have many benefits, including protection against soil erosion and enhancements of water quality, to name a couple, they have also been criticized as they could lead to important decreases in biodiversity, in some instances. Tree breeding programs offer the potential to increase productivity without increasing the area used for plantations.

Tree breeding relies, first, upon the existence of genetic variation in a given trait, such that genotypes with superior performance can be selected. It is also essential to understand how the performance of genotypes varies with environment. Traditionally, a lack of genotype x environment (G x E) interaction has been considered as advantageous because, when there is no G x E, the best-performing genotypes are always the same set of genotypes, regardless of the environment. However, recent studies propose that phenotypic plasticity could be beneficial, as most plastic genotypes have sometimes been documented to be the most productive ones (Aspinwall et al. 2014).

In fact, there are long-standing debates on whether the emphasis of tree breeding programs should be on producing genotypes suitable for specific environments or, on the contrary, on genotypes suited to wide range of environments. It is therefore important to clarify whether genotypes that increase productivity under optimal conditions, but also under stressful conditions can be identified (Aspinwall et al. 2014). Generally, the goal of tree breeding is to produce genotypes that are productive across range of environments or management conditions.

Forest trees and plantations generally increase productivity under eCO₂, although the response is modulated by environmental factors like soil fertility, drought, ozone, to name a few (Spinnler et al. 2003, Kaakinen et al. 2004, Lindroth 2010). While syntheses studies on the effects of CO₂ on production, alone or in interaction with other environmental drivers, such as temperature, are common. Studies on intra-specific variation, however, are scarce and I am not aware of any systematic analysis that integrates across the published literature.

Here, I report the results of meta-analysis on intra-specific responses of tree growth and gas exchange to elevated CO₂. More specifically, I first sought to understand the degree of variation

in response to CO₂ across genotypes, relative to that reported in inter-specific studies. Only under substantial intra-specific variation can a tree breeding program be developed. Second, I aimed at understanding whether there is significant G x E by testing whether the best performing genotypes under eCO₂ can be predicted based on their performance under aCO₂. CO₂ manipulation experiments are expensive, and breeding programs would be more practical if they could be based upon aCO₂ responses. Third, I sought to disentangle the mechanisms underlying genotypic differences in the response of trees to eCO₂. I expected a higher photosynthesis than growth response to eCO₂, indicating an important role for allocation. Finally, I aimed at understanding whether genotypes performing best under eCO₂ would also be least affected by the negative impacts of environmental stressors (drought, warming, ozone). However, as I will describe below in more detail, I could only assess CO₂ x O₃ interactions.

Materials and Methods

Literature survey and data collection

I conducted extensive literature searches in Web of Science and Scopus with the expression “wood growth CO₂” (March 2015). This led to 644 and 444 papers, respectively. However, most of these studies had to be discarded because they dealt with inter-specific comparisons. After selecting all the papers dealing with intra-specific variation, I additionally searched all the references cited within those papers to find more studies. I ended up with a total of 27 papers relevant for this study, which reported intra-specific variation across 8 species (Table 1).

I digitized all the data these papers contained (plot digitizer General License version 2.0, GPLv2) for a total of 15 variables : height, stem biomass, stem volume, photosynthesis, diameter, biomass production, leaf production, leaf area, leaf area index, root dry biomass, total

dry biomass, wood density, leaf biomass, branch biomass, shoot-root dry biomass. I also took note of growing conditions, and only studies conducted under ample water and nutrient supplies were used. 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 because this data was available for 14, 8, 8 and 10 studies respectively.

I originally intended to understand the effects of CO₂ in interaction with other environmental drivers (warming, drought, ozone, etc). However, due to lack of data, we could only assess CO₂ x O₃ interactions, as this was the only additional driver for which a minimum of 11 studies were found.

Meta-analysis

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, I only used the last value per treatment and study, as that ensured plants were at the oldest stage possible. Plant age ranged from 60 days - 7 years, depending on the study. I used the response ratio (RR) to calculate effect sizes because this is a common metric used in ecological meta-analyses (Brook 1999), including those interested in the response to eCO₂ (Hedges et al. 1999, Curtis and Wang 1998). To avoid problems associated with studies not properly reporting sample sizes and variances, I performed unweighted analysis. Confidence intervals (CIs) for effect-size estimates were calculated by bootstrapping the unweighted data with a resampling of 1,000 iterations. I considered eCO₂ had a significant effect when the interval captured by the CI did not cross 0. R statistical software was used in all calculations (R Core Team 2013).

In order to avoid publication bias, that is, “the tendency for results that are statistically significant to be more likely to be published than those that fail to detect significance” (Rosenthal 1979), I calculate Rosenthal’s fail-safe number using the library *metafor* (Wolfgang V. 2010). This analysis indicated that, in the worst case, 868 studies with negative results would have been necessary to have an impact on my results. Further details on how the meta-analysis was done follow Wang et al. (2012). Although calculations were performed on RR, I report the results as % change, for simplicity.

After calculating effect sizes and associated CIs, I assessed whether relative genotype performance at aCO₂ was maintained at eCO₂ by regressing the values at eCO₂ against those at aCO₂ 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 analysis, we conducted a normalization, by means of linear rescaling, so that values would be within the same axis of variation and comparable. To examine rank changes, all genotypes within one study were given a number based on how they ranked in terms of performance (1st, 2nd ...), and regressed the ranking at eCO₂ against the ranking at aCO₂.

To understand the role of C input in driving growth responses, I regressed the response in height, stem biomass and stem volume against the response in photosynthesis. To understand the interaction between CO₂ and O₃, I regressed the response ratio of height and stem volume to eCO₂ against the response ratio to O₃ and also against the response ratio to eCO₂ and O₃. Regression analyses were performed in Sigma Plot (version 12.0, Systat Software Inc., San Jose, USA).

Results

Intra-specific variation in the response to elevated CO₂ growth and photosynthesis across genotypes

Averaged across all studies we observed that tree growth and gas exchange were significantly increased by elevated CO₂ (Fig. 1). However, there were strong differences in the response to eCO₂ across traits. For instance, photosynthesis showed a much larger eCO₂ response (106%) than height (11%), stem biomass (27%) and stem volume (48%). It is also noteworthy that the response of stem volume was almost twice that of stem biomass and almost four times that of height.

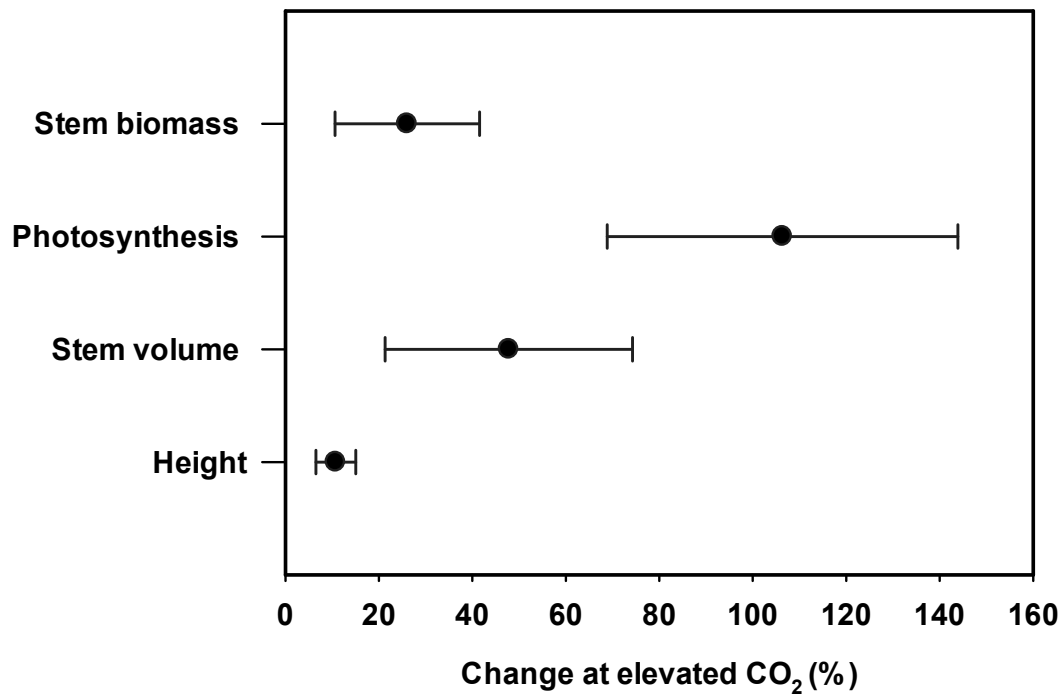


Figure 1. Responses to eCO₂ of stem biomass, photosynthesis, stem volume and height. Each data point represents the mean (\pm 95% CI) from 8 papers for stem biomass, 10 papers for photosynthesis, 6 papers for stem volume and 14 papers for height (Table 1). Significant responses occur the error bars do not cross the 0% line (at $P < 0.05$).

When examining individual studies, I observed that the response to eCO₂ was significant ($P < 0.05$) for photosynthesis, stem volume and stem biomass, and marginally significantly for height ($P < 0.1$; Fig.2).

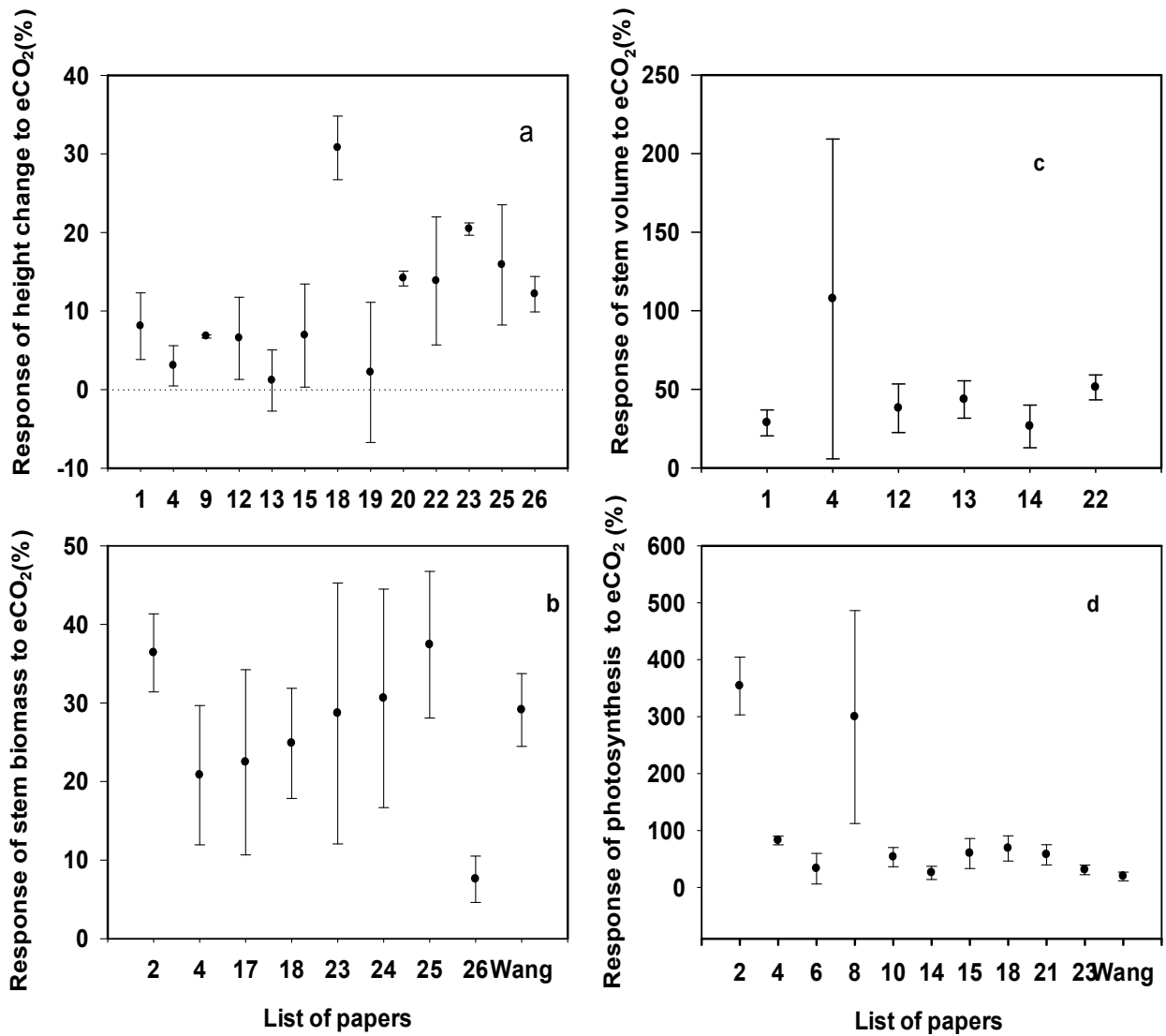


Figure 2. Intra-specific variation in the response to eCO₂ for (a) height, (b) stem biomass, (c) stem volume and (d) photosynthesis for each published study (see Table 1 for information on species and further details for each study). The range in intra-specific variability is indicated by the mean and the 95% CI error 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 eCO₂ (Wang et al. 2012). Y-axis scale differs between panels.

The trait that showed the highest response was photosynthesis (up to 354 %, Fig.2d), while the trait with the lowest response was height (down to -9 % Fig2.a). This result also indicates that photosynthesis has a higher response to eCO₂ than other growth traits. Furthermore, height was the trait with the smallest response to eCO₂, even showing non-significant responses in two studies (papers 13 and 19 in Fig. 2a). Stem biomass and stem volume showed intermediate but always significant responses (Fig.2b, c).

We observed a wide range of variability in response to eCO₂ across genotypes in the different studies, as indicated by the large error bars in Fig. 2. To compare the degree of intra-specific variability with the degree of inter-specific variability, we compared the 95% CI in our results, with those reported by Wang et al (2012) who assessed intra-specific variability. Overall, we observed that, for those traits also reported by Wang et al. (stem biomass and photosynthesis), the degree of intra-specific variation (the length of the error bar in Fig. 2) was, at least, as large as the degree of inter-specific variability.

Table 1. Summary of genotypic variation among clones of different species in response to elevated CO₂ for: height, stem biomass, stem volume, photosynthesis. The symbol represents “Y” =yes, “N” = no and “-” = no data available. Data are taken from 27 separate papers that include *Populus* (18), *Picea sitchensis* (4), *Betula pendula* (3) and *Fagus sylvatica* (2).

| References | Paper number | Species | Number of clones | Height | Stem biomass | Stem volume | Photosynthesis |
|-----------------------------|--------------|---------------------------------------------------|------------------|--------|--------------|-------------|----------------|
| Isebrands et al. (2001) | 1 | <i>Populus tremuloides</i> | 5 | Y | - | Y | Y |
| R. Tognetti et al. (2005) | 2 | <i>Populus deltoides</i> and <i>P. nigra</i> | 2 | - | Y | - | Y |
| Wang et al. (2000) | 3 | <i>Populus tremuloides</i> | 5 | - | - | - | - |
| R.Tognetti et al.(1990) | 4 | <i>Populus deltoides</i> and <i>P. nigra</i> | 2 | Y | Y | Y | Y |
| K.Kostiainen et al.(2005) | 5 | <i>Populus tremuloides</i> | 4 | - | - | - | - |
| Centritto and Jarvis (1998) | 6 | <i>Picea sitchensis</i> | 4 | - | - | - | Y |
| Centritto et al.(1999) | 7 | <i>Picea sitchensis</i> | 4 | - | - | - | - |
| Riikonen et al. (2005) | 8 | <i>Betula pendula</i> | 3 | N | - | - | Y |
| Noormets et al.(2010) | 9 | <i>Populus tremuloides</i> | 2 | Y | - | - | - |
| J.Darbah et al.(2010) | 10 | <i>Populus tremuloides</i> | 2 | - | - | - | Y |
| McGrath et al.(2010) | 11 | <i>Populus tremuloides</i> | 2 | - | - | - | - |
| Kubiske et al.(2007) | 12 | <i>Populus tremuloides</i> | 5 | Y | - | Y | - |
| Noormets et al.(2001) | 13 | <i>Populus tremuloides</i> | 2 | Y | - | Y | - |
| Cseke et al.(2009) | 14 | <i>Populus tremuloides</i> | 2 | - | - | Y | Y |
| J. Biosci et al.(1998) | 15 | <i>Hevea brasiliensis</i> | 2 | Y | - | - | Y |
| Townend et al.(1993) | 16 | <i>Picea sitchensis</i> | 4 | - | - | - | - |
| Riikonen et al.(2004) | 17 | <i>Betula pendula</i> | 3 | N | Y | Y | - |
| Kendall et al.(2003) | 18 | <i>Trembling poplar and Hybrid Aspen</i> | 7 | Y | Y | - | Y |
| Z.-B Luo et al. (2005) | 19 | <i>Populus tremuloides</i> | 6 | Y | - | - | - |
| Centritto et al.(1999) | 20 | <i>Picea sitchensis</i> | 4 | Y | - | - | - |
| Ceulemans et al.(1997) | 21 | <i>Populus deltoides</i> and <i>P. nigra</i> | 2 | - | - | - | Y |
| Ceulemans et al. (1996) | 22 | <i>populus trichocarpa</i> and <i>p.deltoides</i> | 2 | Y | - | Y | - |

| | | | | | | | |
|--------------------------|----|-----------------------------------------|---|---|---|---|---|
| Ceulemants et al. (1995) | 23 | <i>Populus trichocarpa, P.deltoides</i> | 2 | Y | Y | - | Y |
| Spinnler et al.(2003) | 24 | <i>Fagus sylvatica and Picea albies</i> | 8 | - | Y | | |
| Dickson et al.(1998) | 25 | <i>Populus deltoides</i> | 4 | Y | Y | - | |
| Dickson et al.(2001) | 26 | <i>Populus tremuloides</i> | 2 | Y | Y | Y | |
| Wustman et al.(2001) | 27 | <i>Populus tremuloides</i> | 3 | Y | - | - | - |

Genotype x environment interaction in response to eCO₂

Our results showed a strong positive correlation between values at ambient and at elevated CO₂ for all traits (Fig. 3). In fact, the R² of the regression between normalized photosynthesis, stem biomass, stem volume and height values measured at eCO₂ vs. normalized values measured at aCO₂ was always above 0.65 (Fig. 3).

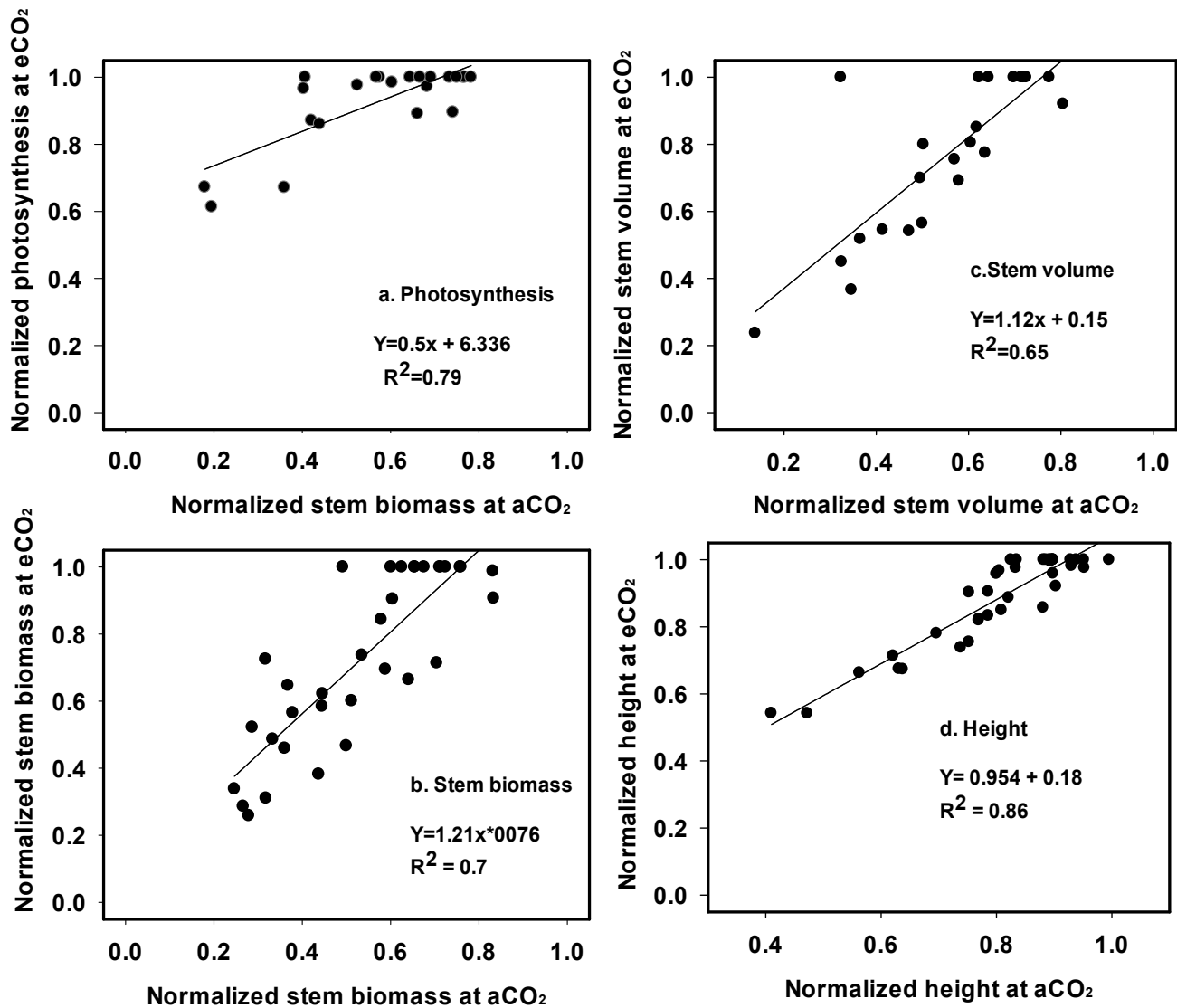


Figure 3. Comparison of genotype values at elevated vs. ambient CO₂ for normalized (a) photosynthesis, (b) stem biomass, (c) height and (d) stem volume. Each data point represents the value of a given genotype in a given study.

There was a significant and positive relation between the genotype ranking at ambient and at elevated CO₂ concentration for height, stem volume and stem biomass ($P < 0.01$). That is, genotypes maintained their level of performance after exposure to eCO₂, relative to the performance of the other genotypes. However, the genotype ranking for photosynthesis at aCO₂ was not predictive of genotype ranking at eCO₂ ($P = 0.24$). It is important to note that genotype rankings only considered the mean (and not the error) value for a genotype. That is, the trait value for a genotype ranked as 1 will be higher than for a genotype ranked as 2, but, the difference may not be statistically significant.

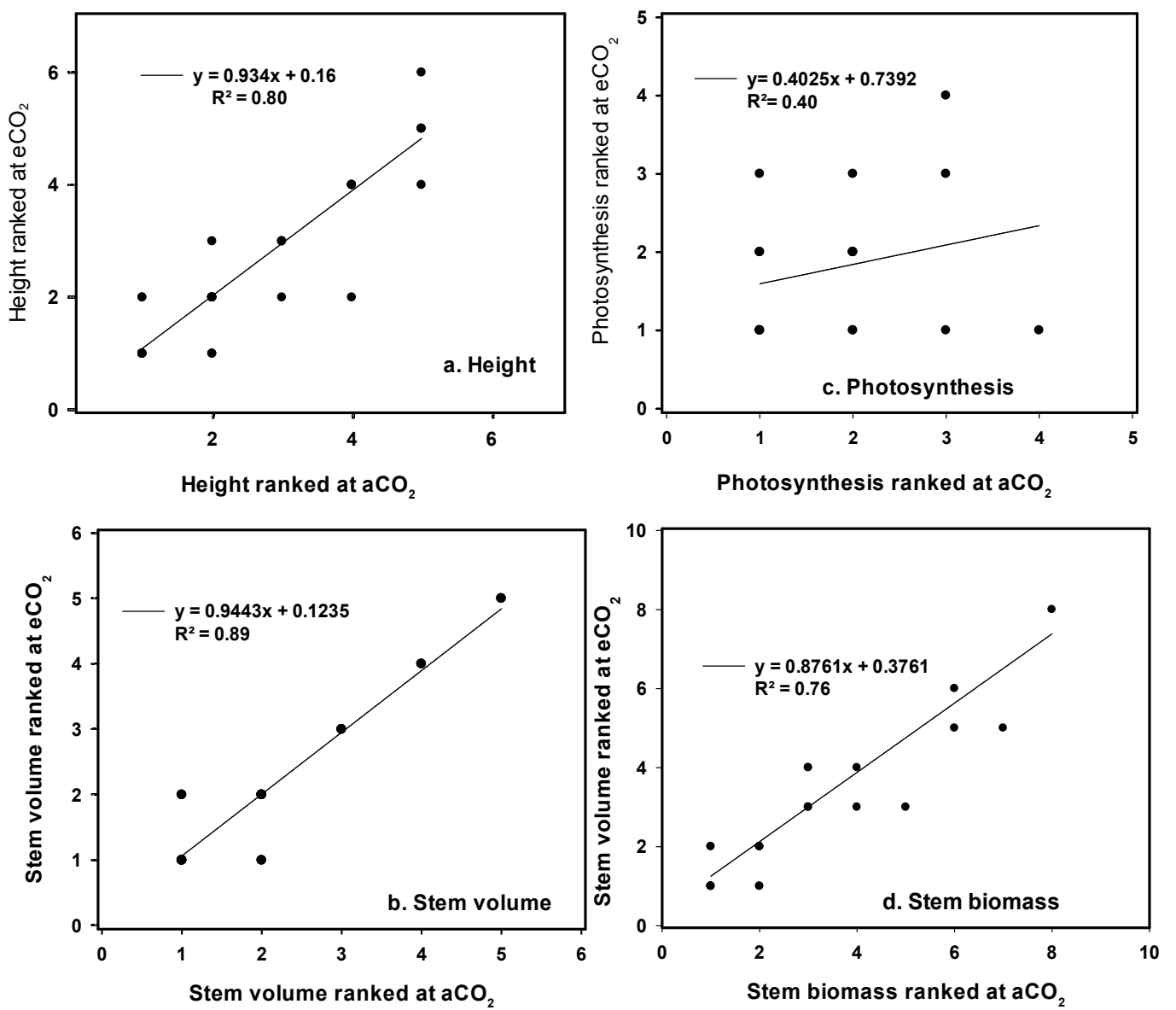


Figure 4. Comparison of genotypes grown in ambient CO₂ to elevated CO₂ height (a), stem volume (b), photosynthesis (c) and stem biomass (d) after ranking of genotypes of the same species were done. Y-axis scale differs between panels.

Photosynthesis as a driver the growth response

Photosynthesis always showed a higher response to eCO₂ than height, stem biomass and stem volume (Fig. 1, Fig. 2). The response of photosynthesis to eCO₂ was significantly correlated with the responses of stem biomass to eCO₂ ($P > 0.022$, $R^2 = 0.59$), but not with the response of height ($P = 0.41$; Fig. 5). I also attempted to correlate photosynthesis with stem volume, but only 4 data points were available (not all papers that measured photosynthesis also measured the other growth traits) and no meaningful correlation could be extracted.

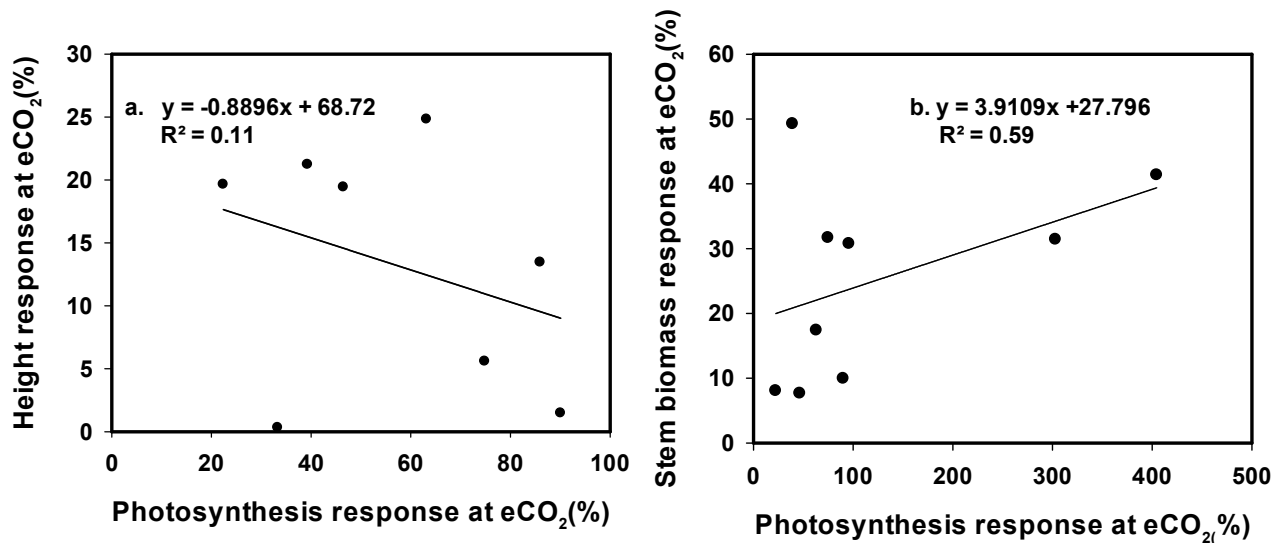


Figure 5. Comparison of the response to eCO₂ in photosynthesis against height (a) and against stem biomass (b). Data points represent the percent change response of each clone in those studies jointly reporting photosynthesis and height and photosynthesis and stem biomass (b). Relationships between photosynthesis and stem volume could not be examined due to a lack of data. Y-axis scale differs between panels.

Effects of CO₂ x O₃ on genotype growth and photosynthesis

In response to O₃, I generally observed reductions in height, photosynthesis and stem biomass and volume. However, genotypes growing under O₃ plus elevated CO₂ experienced increases in height, stem biomass, stem volume and photosynthesis (Fig. 6 and 7). I observed that the response to O₃ was not correlated with the response to CO₂ (Fig. 6). However, there was a significant correlation between the response to CO₂ and the response to CO₂+O₃ (Fig. 7)

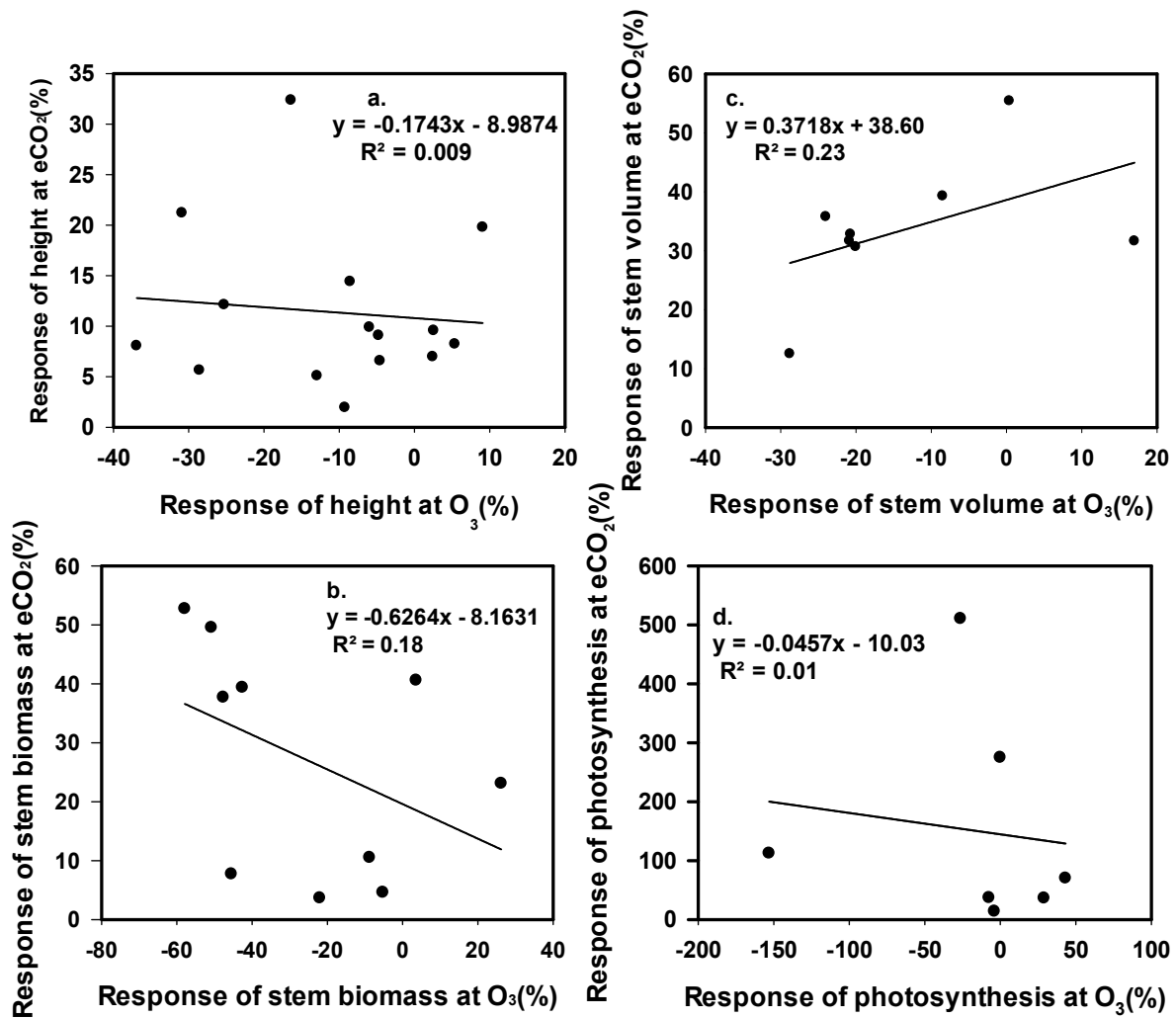


Figure 6. Comparisons of the % change for variables: Height change at elevated CO₂ to height change at O₃ (a), stem biomass change at elevated CO₂ to stem biomass change at O₃ (b), stem volume change at elevated CO₂ to stem volume change at O₃ (c) and, photosynthesis change at elevated CO₂ to photosynthesis change at O₃ (d). Data points represent the percent change of each clone taken from common separate paper (4 paper for height, 3 paper for stem biomass, 3 paper for stem volume and 3 paper for photosynthesis). Y-axis scale differs between panels.

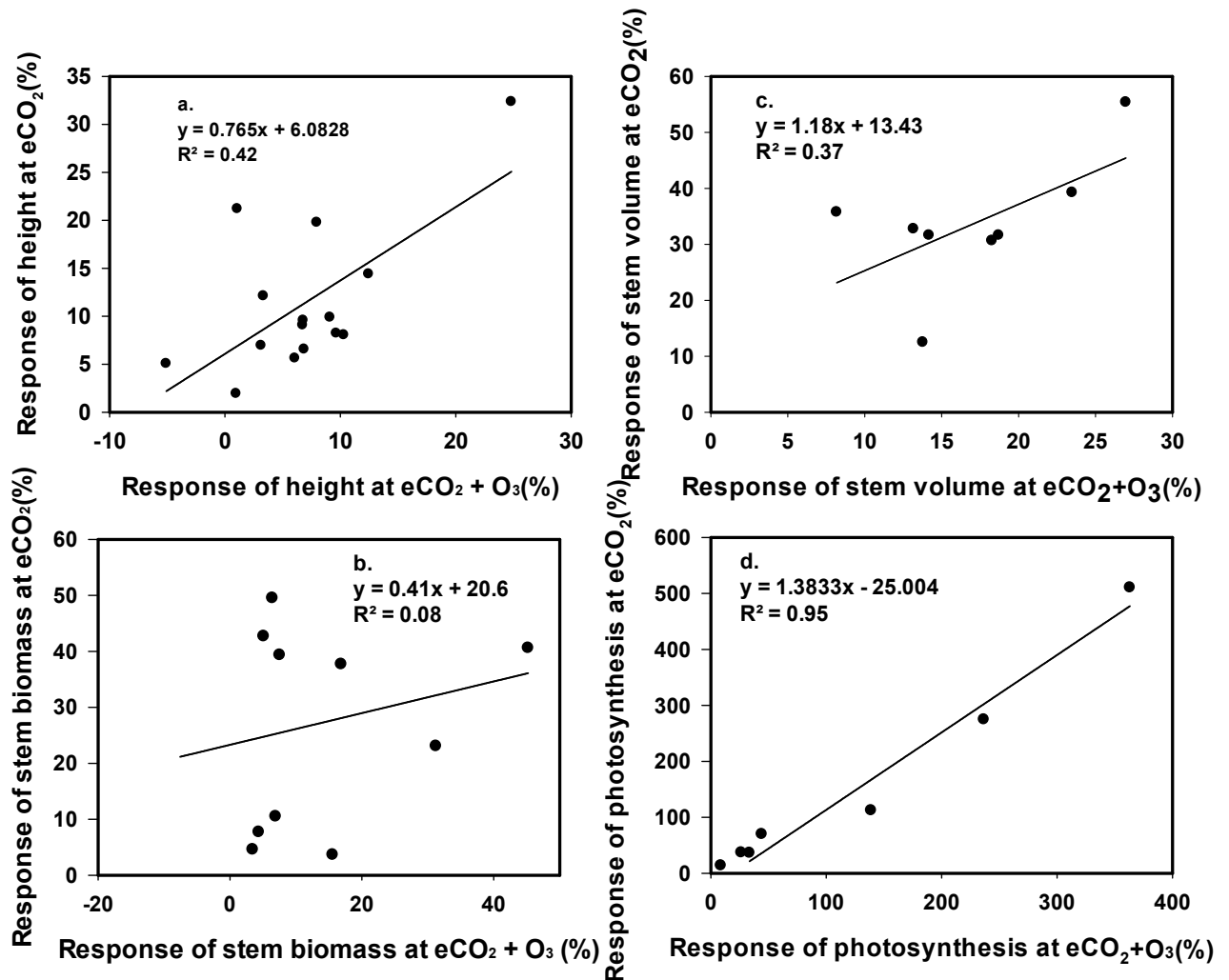


Figure 7. Comparisons of the % change for variables: Height change at elevated CO₂ to height at eCO₂+O₃ (a), stem biomass change at elevated CO₂ to stem biomass change at eCO₂ + O₃ (b), stem volume change at elevated CO₂ to stem volume change at eCO₂ + O₃ (c) and, photosynthesis change at elevated CO₂ to photosynthesis change at eCO₂ + O₃ (d). Data points represent the percent change of each clone taken from common separate paper (4 paper for height, 3 paper for stem biomass, 3 paper for stem volume and 3 paper for photosynthesis). Y-axis scale differs between panels.

Discussion

Genotype variation in response to elevated CO₂

Elevated CO₂ had a significant effect on tree growth and gas exchange, but the magnitude of the response differed between species and genotypes. The increase in stem biomass I observed was of similar magnitude to those commonly reported in meta-analyses 29 % examining inter-specific variation (Wang et al. 2012) and many other finding also show consistent results (Norby et al. ,2005,Körner 2006, Hedges et al. 2007, Cseke et al. 2009, Ainsworth et al. 2015). However, I observed an increase in photosynthesis response (106%) much larger than those typically documented in Wang (19 %) (Fig.2). This could be partly because of the dominance of more-responsive juvenile trees in this study (mostly between 6 month to 3 years) as it has been previously documented (Wang 2007, Rasineni et al. 2011, Kostianen et al. 2014). This could also be because photosynthesis is a short-term response, with larger temporal fluctuations than other processes, like growth, which integrate responses over longer time-scales. This large photosynthesis response could also be a result arising from the relatively number of published studies on intra-specific variation, which overall limits the degree of generalization from these results. In fact, most of the studies analyzed here were conducted in *Populus*, so further research is needed to understand genetic variation in response eCO₂ in species from other genus.

We observed a large degree of genetic variation in growth and its response to eCO₂. Indeed, the range in intra-specific variation we observed within the different studies was generally of the same order of magnitude as the variation documented in meta-analyses on inter-

specific variation (Fig. 2). These results indicate that there is potential for selecting eCO₂-responsive genotypes for plantations.

Lack of interactions between genotype and environment in response to eCO₂

We did not find evidence for significant G x E interactions. The values of growth traits and photosynthesis at eCO₂ could be predicted from the values at aCO₂ and, additionally, the relative genotype rankings for growth at aCO₂ were maintained also at eCO₂. Overall, these results indicate that selecting best-performers under current conditions should also lead to selecting the best performers under eCO₂.

Carbon allocation, and not photosynthesis, was the major driver of growth responses

Based on my meta-analysis in all study papers I considered, I found that the most CO₂ responsive variable was photosynthesis. However, relationships between photosynthesis and growth were not so clear. First of all, the response of photosynthesis was much larger than that of growth. It is important to note that photosynthesis is reported here at the leaf, and not at the individual level. It is likely that trees growing under eCO₂ had higher leaf area than trees growing at aCO₂ such that the response of total plant photosynthesis to eCO₂ would have been much larger than the leaf response reported here. It thus seems like there was a mismatch between C input and aboveground growth, as the former seemed to be much larger than the latter.

Relationships between the response of photosynthesis and the response of growth were not always significant. Indeed, I observed no significant relationship between photosynthesis and height. Moreover, while photosynthesis always a positive response to eCO₂, height did not always have a positive response and, sometimes, the response was even negative. However, the response of stem biomass was always positive. We did not have enough data to examine responses of secondary growth but, overall our results suggest that the positive growth response was more driven by changes in stem diameter than in stem height.

Considering the increase in C input was much larger than in growth, and that primary growth showed a particularly small response to eCO₂, we can conclude that, while CO₂ may have a positive effect on stem biomass, that may not necessarily translate into a height increase. In fact, our results point towards increases in allocation of Carbon belowground as the main response to eCO₂.

It is important to 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 CO₂ under limited nutrients could exacerbate this response.

Tree growth response to ozone and ozone plus CO₂ concentration -

It has been well documented that exposing plants to ozone often decreases photosynthesis and stomatal conductance (Nunn et al. 2005, Riikonen et al. 2005). Ozone has more effect on photosynthesis, carbon fixation, light usage efficiency and the exposure to plants is a loss in the

amount and activity of Rubisco (Noormets et al. 2001) and its consequence will be a reduction in photosynthetic capacity.

In my study, in response to O₃, height, stem biomass, stem volume and photosynthesis all had a showed a negative response.

This result is consistent to the experimental results that showed elevated ozone decreases chlorophyll concentration (Isebrands et al. 2001, Körner 2006). Another evidence showed also ozone caused a decrease in leaf nitrogen content and photosynthetic ability and also it has damaging physiological responses to plants (Kostiainen et al. 2014, Noormets et al. 2010, Dickson et al. 2001).

From the result I suggest that increase of ozone in the future climate will be one of environmental factor that constrains tree growth, although the combination of ozone and eCO₂ might overcome the ozone's effect. . In fact, genotypes grown under O₃ + eCO₂ showed a positive response, indicating that the positive effect of CO₂ may overcome, at least to some extent, the negative effects of O₃. This result additionally indicates that, by selecting genotypes most responsive to eCO₂, we are concomitantly selecting those most responsive to eCO₂+O₃.

To the contrary, for plants grown in ozone plus elevated CO₂, enormous experimental research findings agree to our result and demonstrate that since elevated CO₂ generally increases carbon fixation and decreases stomatal conductance, increasing CO₂ concentration alleviate the negative effect of O₃ on plant growth and consequently will have a positive response (Dickson et al. 2001, Riikonen et al. 2005, Kubiske et al. 2007, Noormets et al. 2010).

In general, the increase in response to elevated CO₂ and ozone plus eCO₂ and the negative response to ozone concentration which is seen in different tree species has important inference in planning plantations in future program.

Conclusion

In conclusion, in this study I observed that:

- There is significant genetic variation in the response of growth to eCO₂.
- Genotype growth under eCO₂ is highly predictable based on genotype growth at aCO₂.
- Large increases in photosynthesis do not lead to aboveground growth increases of comparable magnitude.
- Increases in primary growth in response to eCO₂ are relatively smaller than in stem biomass.
- Increasing C allocation belowground seems to be the main primary fate of enhanced photosynthetic rates.
- Exposing trees to O₃ reduce tree growth but eCO₂+O₃ have significant positive effect on tree growth.
- Further research should address the effects of CO₂ in interaction with other global change agents and in species belonging to a genus different from *Populus*.

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