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Growth and yield stimulation under elevated CO₂ and drought

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Review

Growth and yield stimulation under elevated CO₂ and drought: A meta-analysis on crops Casper J. van der Kooi^{a,b,*}, Martin Reich^{b,1}, Markus Löw^c, Luit J. De Kok^{a,b}, Michael Tausz^a^a Department of Ecosystem and Forest Sciences, The University of Melbourne, Creswick (VIC), Australia^b Laboratory of Plant Physiology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Groningen, The Netherlands^c Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Creswick (VIC), Australia

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ABSTRACT

Exposure of plants to elevated CO₂ (eCO₂) has a number of physiological effects, including increased photosynthetic carbon fixation and decreased stomatal conductance, resulting in greater growth and yield and also improved water use efficiency. Therefore, eCO₂ is considered to ameliorate the adverse effects of drought. To test this assumption in existing literature, we undertook a meta-analysis to study the interactive effects of eCO₂ and experimentally imposed drought on biomass and yield of crops and pasture grasses. We found that for crop species with C₃ metabolism, eCO₂ stimulates biomass accumulation and yield under both well-watered and dry conditions to a similar extent. For C₄ crops, however, stimulation of biomass accumulation and yield by eCO₂ occurs only under dry growing conditions. Disentangling other functional groups (annuals vs. perennials, crop species, experimental setups) also showed that plant metabolism greatly determines the overall effect of eCO₂ and water availability on plant performance. Our results suggest that crops grown in areas with limited water availability will benefit from future eCO₂, regardless of their metabolism. Drought leads to stomatal limitation of photosynthesis in both C₃ and C₄ crops, which is alleviated when the plants are grown under eCO₂.

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1. Introduction

Atmospheric CO₂ concentrations have increased since the onset of the industrial revolution and are expected to further increase, together with more frequent periods of low water availability (IPCC, 2014). Drought stress is a major constraint to crop yield (Nemani et al., 2003; Cattivelli et al., 2008; Farooq et al., 2009) and quality (Peterson et al., 1992; Champolivier and Merrien, 1996; Singh et al., 2008; Vandegeer et al., 2013). Therefore, in order to maintain future food security, knowledge about the interactive effects of elevated CO₂ (eCO₂) and drought stress on crop performance is of great importance (Leakey et al., 2006; Li et al., 2009; Aljazairi et al., 2015). In many regions climate change will not only lead to enhanced drought stress via reduced rainfall but also to lower air humidity due to higher temperatures, leading to an increased leaf-to-air vapor pressure deficit (Lobell et al., 2014).

Numerous studies dealt with effects of eCO₂ (e.g., Kimball, 1983; Cure and Acock, 1986; Lawlor and Mitchell, 1991; Jablonski et al., 2002; Ainsworth and Long, 2005; Högy et al., 2009; Reich et al., 2015) or drought stress on plant performance (e.g., Blum, 2005; Rampino et al., 2006; Sadras, 2009; Grantz et al., 2015; Taeger et al., 2015; Wujeska-Klaue et al., 2015). Fewer studies addressed the complex interactive effects of eCO₂ and drought stress. Most studies that examined the effect of low water availability in combination with eCO₂ concluded that eCO₂ leads to decreased plant transpiration, and therefore eCO₂ is expected to ameliorate the adverse effects of drought stress (Kirkham et al., 1991; Wall et al., 2001; Wall, 2001; Ainsworth and Rogers, 2007; Tausz-Posch et al., 2013; Kirkham 2011; Kadam et al., 2014; Miranda-Apodaca et al., 2015).

Plants with different mechanisms of carbon fixation are expected to respond differently to eCO₂ and drought. In terms of stimulation of photosynthesis and growth, C₄ plants generally respond less to eCO₂ than C₃ plants (Poorter and Navas, 2003). This difference is due to the efficient way of CO₂-fixation of C₄ plants, which have a cellular mechanism to increase CO₂-concentration in the bundle sheath cells where the carbon fixing enzyme rubisco is located (Sage et al., 1999; Hatch, 2002; Poorter and Navas, 2003; Ainsworth and Long, 2005). Because of this cellular carbon concentrating mechanism, C₄ plants are able to reduce their stomatal conductance to a minimum, especially when water loss is high. This leads to a higher tolerance to drought of C₄ over C₃ species (Pearcy and Ehleringer, 1984; Sage et al., 1999; Dias and Brüggemann, 2010; Alfonso and Brüggemann, 2012) and to different CO₂-response curves of photosynthesis (Farquhar et al., 1980).

Drought and eCO₂ have separate as well as combined effects on plant biomass and yield (Morison and Gifford, 1984; Tyree and Alexander, 1993; Ziska et al., 1997; Wullschlegel et al., 2002; Nelson et al., 2004; Leakey et al., 2009; Kirkham 2011; Kadam et al., 2014). Biomass is expected to increase under eCO₂ due to greater carbon fixation and due to a prolonged active growing period by maintaining the soil water reserves for longer (e.g. Reyes-Fox et al., 2014). An extended period of grain filling under eCO₂ may subsequently increase crop yield, because the efficiency of carbon-allocation to grains and the filling period duration affect yield quantity (Daynard et al., 1971; Nass and Reiser, 1975; Tollenaar and Lee, 2006; Kirkham 2011). Though, whereas biomass production is the direct result of photosynthetic carbon assimilation and water uptake, the formation of yield is a more complex process with additional physiological mechanisms involved (Yoshida, 1972; Jenner et al., 1991; Tollenaar and Lee, 2006; Hawkesford et al., 2013). Hence, the effects of eCO₂ and drought on biomass and yield might not be identical (Poorter, 1993).

A meta-analysis is a powerful tool to quantitatively evaluate the overall pattern across many independent studies because it combines the results into one measure: the effect size (Glass,

1976 reviewed by Nakagawa and Cuthill, 2007). Meta-analytical studies on the interactive effects of eCO₂ and stomatal conductance or seasonal drought have been conducted (e.g., Morgan et al., 2004; Ainsworth and Rogers, 2007; Leakey et al., 2009; Norby and Zak, 2011); however, the interactive effects of eCO₂ and experimentally imposed drought (i.e., studies that simultaneously applied at least two water treatments) have only been touched on briefly in meta-analyses (e.g., Ainsworth and Long, 2005), or considered only C₃ crops (Bishop et al., 2014).

We focused on plant species that are important for agricultural uses, i.e., crops and pasture grasses, for different reasons. Identifying the effects of global change on crop growth and yield is essential in order to maintain future food security and crops can serve as an exemplary model for other plant species. From a quantitative perspective, crops are particularly suitable because many experimental studies examined the interactive effects of eCO₂ and drought in this group of species, allowing comparisons between species and functional groups. For our meta-analysis, we analyzed studies that juxtaposed an experimental drought treatment with well-watered controls in interaction with exposure to elevated and ambient CO₂. We evaluated the differences between functional groups such as C₃ and C₄ or annual and perennial crops. Where sufficient data were available, also the differences between important crop species and between different experimental setups were studied. We aim to test the hypothesis that crops and pasture grasses benefit more from eCO₂ under drought than under well-watered conditions.

2. Materials and methods

2.1. Data collection

The studies included in our meta-analysis were obtained by systematically searching Google Scholar using the following search terms in various combinations: “crops”, “drought”, “elevated CO₂”, “interaction”, “interactive effects”, “moisture”, “stress” and “water availability”. For biomass, we extracted 62 data entries (for both dry and well-watered conditions) from 41 different experimental studies on 30 crop species (Table S1). For yield, we extracted 19 data entries (for both dry and well-watered conditions) from 17 experimental studies on 8 crop species (Table S2). The included studies were published in scientific literature between 1979 and 2014. We crosschecked references used in and to all studies incorporated in our analysis. For all obtained scientific papers we examined the experimental setup, the statistics applied and whether the study reported all necessary parameters and variables (see below).

Some experiments on eCO₂ and drought stress simultaneously tested a third variable. For these studies we only used the results corresponding to the ‘ambient’ situation for that third variable. For example, in a three-way interaction including temperature, only values for ambient temperature were used in our analysis. Only experiments with continuous or progressing dry and well-watered treatments were included and the few available studies with alternating dry-well-watered cycles were left out, as their number was too small to evaluate differences to continuous treatment protocols. In defining the “wet” and “dry” treatments, we followed the definition of the authors of the original experiment; the effects of severity of the drought stress were also evaluated (see below). Where biomass and yield data were reported at multiple time points during the season, we used the final measurements in our analysis, as the number of papers reporting continuous data is too small for an in-depth study.

For studies that did not report a measure of variance (standard deviations, standard errors, or variances), the given *p*-values or significance levels were converted to standard deviations (following: Higgins and Green, 2011; Wujeska et al., 2013). Where exact *p*-

values were not given, significance levels were considered 0.1% smaller than the given α -value (e.g., if the study reported significant results at the 0.05 level, then the p -value was considered 0.049); non-significant values were considered as 0.99 (following: Wujeska et al., 2013).

2.2. Data analysis

To measure the effect size, we used the log-transformed ratio of means, also called the log response ratio. The ratio of means is given by the formula: $R = \bar{X}_E / \bar{X}_C$ where $\bar{X}_E$ and $\bar{X}_C$ are the means for the experiment and control, respectively. However, the sample distribution of R is skewed and R is affected more by changes in the denominator than by changes in the numerator, especially when the denominator is small (Hedges et al., 1999). Log transformation, via $L = \ln(R) = \ln(\bar{X}_E) - \ln(\bar{X}_C)$, provides a more normal sample distribution, treats changes in the nominator and denominator equally and is therefore more suitable (Curtis and Wang, 1998; Hedges et al., 1999). Indeed, the log-transformed ratio of means is a common metric used in studies on eCO_2 (e.g., Curtis and Wang, 1998; Hedges et al., 1999) and it yields straightforward results, because it can easily be transformed to a proportional difference.

We calculated the effect sizes using the metafor package in R (Viechtbauer, 2010; Core Team, 2012) and greater weight was assigned to experiments with greater precision, i.e., smaller variances and/or greater sample sizes (Curtis and Wang, 1998; Hedges et al., 1999). We calculated the effect sizes for eCO_2 relative to ambient CO_2 , while incorporating water regime as a factor. In addition, the degree of water supply, the CO_2 -enrichment method applied in the experimental setup and various plant characteristics were included as modifying factors. Only effect sizes that represented at least three experimental studies are shown, i.e. only effect sizes based on at least three publications are presented. Because there were fewer studies that reported yield, the modifying factors 'crop species', 'plant life form', 'water regime' and ' CO_2 -enrichment method' were only tested for biomass.

3. Results

Many experiments assessed crop performance under either eCO_2 or drought stress, but fewer studied the effects of eCO_2 and drought simultaneously. The data from 41 studies on crop biomass and 17 studies on crop yield under different CO_2 and watering regimes were incorporated in our analysis. The sample sizes shown in the figures and in the text indicate the number of unique experimental studies that were used to calculate an effect size, and therefore the number of plant species and cultivars that actually represent an effect size is often higher (see Table S1, S2).

When results from well-watered and dry growing conditions were pooled, crops grown under eCO_2 showed an average increase in biomass of $28.2 \pm 4.3\%$ and an average increase in yield of $26.4 \pm 8.2\%$ (mean $\pm$ 95% confidence interval). Disentangling dry and well-watered growing conditions showed that the eCO_2 effect on biomass accumulation was significantly greater under dry conditions (Fig. 1a; $p = 0.008$; $n = 41$). A similar trend for yield was not significant (Fig. 1b; $p = 0.12$; $n = 17$).

3.1. C_3 and C_4 metabolism

It became clear that C_3 and C_4 crops respond differently to eCO_2 under dry than under fully watered growing conditions, when their effect sizes were analyzed separately. C_3 plants grown under eCO_2 showed increased biomass accumulation and yield in both dry and well-watered conditions (Fig. 2). By contrast, for C_4 plants the eCO_2 effect on biomass accumulation and yield was restricted

Fig. 1. The effect of eCO_2 on crop biomass (a) and yield (b) under dry ("Dry") and well-watered ("Wet") growing conditions. Data are mean effect sizes (log-transformed ratio of means) and 95% confidence intervals; the number of experimental studies representing an effect size is given in parentheses.

to dry conditions. C_4 crops grown under dry conditions showed an approximate 25% increase in biomass accumulation under eCO_2 (Fig. 2a; $p = 0.08$; $n = 13$), whereas the increase was absent under well-watered conditions. Similarly, C_4 plants showed an approximate 30% increase in yield under eCO_2 when grown under dry conditions ($p = 0.04$; $n = 4$), whereas there was no effect of eCO_2 under well-watered conditions (Fig. 2b).

The differences between C_3 and C_4 crops remained when they were pooled per life form, i.e., annuals vs. perennials. In annual C_4

Fig. 2. The interactive effects of eCO_2 and drought stress on crop biomass (a) and yield (b) for C_3 and C_4 plants. Data are mean effect sizes (log-transformed ratio of means) and 95% confidence intervals; the number of experimental studies representing an effect size is given in parentheses.

crops the increase in biomass accumulation could only be observed under dry growing conditions, but in perennial C_4 crops a similar trend was not significant (Fig. 3a). For both C_3 annuals and perennials the increase in biomass accumulation was similar under dry and wet growing conditions (Fig. 3a).

The effect sizes per species confirmed that differences are mainly due to the type of metabolism. The C_3 crop wheat (*Triticum aestivum*) and C_3 pasture grasses showed increased biomass accumulation under both well-watered and dry growing conditions (Fig. 3b). Among the C_4 crops, corn (*Zea mays*), sorghum (*Sorghum bicolor*) and C_4 pasture grasses, the growth stimulation under eCO_2 was apparent under drought and virtually absent in well-watered conditions. The differences between functional groups and species did not change if studies on rice—recognizing rice may be a special case with regard to water use—were excluded (not shown), likely because only two studies on rice are included in our analysis (Table S1, S2). Leguminous crops tended to increase biomass under both well-watered and dry conditions more than non-leguminous plants (Fig. S1), but the limited number of experiments on legumes leaves this result inconclusive.

3.2. Experimental methodologies

To determine the connection between the effect of eCO_2 and the degree of drought, we separated the studies according to the different levels of water supply used in the different experimental setups. We found no significant differences between different watering treatments applied (Fig. 4a). For all water regimes the increase in biomass was on average higher in dry than in well-watered conditions, but this was not significant in these groupings.

For all different CO_2 -enrichment techniques, the effect of eCO_2 on biomass accumulation tended to be larger under dry than well-watered conditions, albeit not significant in these groupings (Fig. 4b). The effect sizes of growth chambers, open top chambers, SPAR chambers and glasshouse experiments were rather variable (Fig. 4b), and were represented by either equal proportions of C_3/C_4 species (open top chambers) or more C_3 species (all other methods; Table S1). The set of FACE experiments analyzed here mostly involved C_4 species (Table S1) and produced less variable results (<10%; see Fig. 4).

4. Discussion

4.1. Effects of drought and elevated CO_2 on biomass and yield

This meta-analysis summarized experiments that addressed the interactive effects of eCO_2 and water supply on biomass and yield of crops and pasture grasses, to test the hypothesis that plants

benefit more from eCO_2 under drought. We analyzed 62 data entries on biomass (for both dry and well-watered conditions) from 41 different experimental studies (Table S1) and 19 data entries on yield (for both dry and well-watered conditions) from 17 experimental studies (Table S2), and found that biomass and yield responded very similarly to eCO_2 (Figs. 1 and 2). The stimulating effect of eCO_2 under drought not only increases biomass accumulation, it also translates into higher crop yield (Fig. 2; see also Ainsworth et al., 2008) which indicates a close relationship between an increase in photosynthesis (and consequently biomass production) and yield (Campbell and Kondra, 1978; Zelitch, 1982; Gebeyehou et al., 1982; Gupta et al., 2001; Murphy et al., 2008). Nevertheless, this can only be said for the current dataset and we do not wish to dispute that biomass and yield should be regarded as independent parameters of plant production.

The results of this meta-analysis showed that eCO_2 increased both biomass production and yield by about 20% and 30% for well-watered and dry conditions, respectively (Fig. 1). Although the approximate 10% difference is not significant, this finding is well in line with reported magnitudes from other synthesis papers (Kimball, 1983; Poorter, 1993; Ainsworth and Long, 2005). Hypothesized differences in the effect of eCO_2 on growth and yield are based on the known stimulation of plant water use efficiency under eCO_2 (see the Introduction), and because crop water use is linked with soil water relations, soil type is an important factor to consider. It is as yet unexplored to which extent the effect of eCO_2 on plant water use efficiency varies among crops and soil types. Only few studies have addressed these issues up to date and therefore many studies included in the database assembled for this present analysis did not report sufficient information to quantitatively address these questions here (see Table S1, S2).

4.2. C_3 and C_4 crops

The data on C_3 crops analyzed in this study suggested that the effect of eCO_2 on biomass and yield is similar between well-watered and dry conditions, whereas C_4 crops respond to eCO_2 only under drought (Fig. 2). For yield, the effect sizes for dry conditions were rather variable, possibly due to the lower sample sizes for yield than for biomass. In addition to low sample sizes, the variability of dryness in experimentally imposed dry treatments might affect the variability of the effect size. Whereas plants growing under fully watered conditions generally receive ample irrigation, dry treatments can vary in their degree of dryness, which might result in higher variability when these different

Fig. 3. Effect of eCO_2 and drought stress on biomass with crops being grouped into C_3/C_4 annuals and perennial (a) and species (b) data are mean effect sizes (log-transformed ratio of means) and 95% confidence intervals; the number of experimental studies representing an effect size is given in parentheses. In (b) only species are included for which data from at least three different studies was available.

Fig. 4. The effects of eCO₂ and drought stress on crop biomass for different experimental setups. (a) The effects per water regime; 20–39%, 40–59% or 60–79%: the amount of irrigation drought stressed plants received, relative to the control plants; certain day: irrigation to drought stressed plants was stopped after a certain day in the experiment; other: drought stress was determined via a different way (e.g., drought stressed plants were only irrigated by rain, whereas control plants would be supplementary irrigated). (b) The effect sizes plotted for different CO₂-enrichment techniques; FACE: free air CO₂ enrichment; GH: glasshouse; GC: growth chamber; OTC: open top chamber; SPAR: SPAR-chamber. Data are mean effect sizes (log-transformed ratio of means) and 95% confidence intervals; the number of experimental studies representing an effect size is given in parentheses. In both graphs only results represented by at least three different experimental studies are plotted.

treatments are pooled, as was the case here (for further discussion on this topic, see §4.3).

The difference between C₃ and C₄ crops was also clear when plotted separately per life form or species (Fig. 3), albeit the differences per species were supported by less statistical power due to low sample sizes. Annual and perennial C₃ crops responded similarly to eCO₂ under both dry and well-watered growing conditions, whereas annual C₄ crops responded to eCO₂ only under dry growing conditions (Fig. 3a). Despite the general assumption that fast growing plant species respond more to eCO₂ than slow growing ones (Poorter, 1998; Poorter and Navas, 2003), the effect of carbon metabolism type *per se* might be greater (Fig. 3a). Analogously, species-specific responses were greatly determined by the plant's carbon fixation metabolism. Crop species with C₃ metabolism (wheat and C₃ grasses) showed a similar increase in biomass production under eCO₂ regardless of the watering conditions, whereas C₄ species (sorghum, corn and C₄ grasses) responded only under dry conditions (Fig. 3b). The results on C₄ crops and pasture grasses presented here are in accordance with previous studies that found a stimulating effect of eCO₂ on C₄-crops only under drought (Leakey et al., 2006; Markelz et al., 2011; Manderscheid et al., 2014). For instance, two studies on C₄ crops (maize and sorghum) demonstrated that eCO₂ delayed the decrease of stomatal conductance and subsequently ameliorated the negative effects of drought stress on photosynthesis via sustaining the intercellular CO₂ concentration (Allen et al., 2011; Markelz et al., 2011).

For C₃ species, eCO₂ seems to increase biomass accumulation of plants under dry conditions to an extent that is nearly identical to the response under well-watered conditions (Figs. 2 and 3). Thereby our results seem to contradict the hypothesis that C₃ species also benefit more from eCO₂ under drought than well-watered conditions (Wullschleger et al., 2002; Morgan et al., 2011; Bishop et al., 2014). The absence of an interactive effect of eCO₂ and drought for C₃ crops can be due to only minor additional stomatal closure caused by eCO₂ compared to the extent of stomatal closure already present due to (severe) drought.

4.3. Different watering regimes and experimental methods

An interactive effect of eCO₂ × drought would be found under milder drought stress when soil drying is delayed by decreased transpiration (Volk et al., 2000; LeCain et al., 2003; Burkart et al., 2011). Higher soil moisture levels are often observed in vegetation

grown under eCO₂, relative to ambient CO₂, as a result of lower plant transpiration (Niklaus et al., 1998; Nelson et al., 2004; Ainsworth and Rogers, 2007). This is in accordance with the recently reported negative correlation between the response to eCO₂ and precipitation, that is, plants were found to respond less to eCO₂ in areas where precipitation was high, relative to dry areas (Hovenden et al., 2014). We found no relationship in response of biomass to eCO₂ when the effect sizes were grouped for the degree of water supply (Fig. 4a), which might be due to the pooling of C₃ and C₄ species. Indeed, the variances seemed smaller when the effect sizes per degree of water supply were plotted separately for different metabolisms (not shown), but there are currently too few studies to allow an in-depth analysis. Possibly the pooling of C₃ and C₄ crops also determined the large variances between different experimental setups. The CO₂-enrichment method applied did not affect the overall effect on biomass production, but variances differed between experimental setups. Interestingly, free air CO₂ enrichment (FACE) experiments showed the lowest variability (Fig. 4b). This can be due to a relatively large number of C₄ plants used in the FACE studies (Table S1), or because of the differences in experimental setups between FACE and non-FACE experiments (Ainsworth et al., 2008), e.g., non-FACE studies use a wider range of eCO₂ concentrations.

4.4. Relative importance of metabolic inhibition and stomatal closure

It was previously assumed that C₄ plant species would lose their relative advantage compared with C₃ species, because only C₃ species would benefit from eCO₂ (Bazzaz, 1990; Bowes, 1993). Later research corrected this view (e.g., Ward et al., 1999; Kadam et al., 2014; Manderscheid et al., 2014), and our meta-analysis showed that under drought C₃ and C₄ crops indeed respond very similarly to eCO₂. Given C₄ crops grown under dry conditions and eCO₂ increase their biomass and yield (Figs. 2 and 3), drought likely impairs photosynthesis of C₄ plants more than often was assumed. We argue that this impairment is initially likely to be largely caused by stomatal closure, rather than by metabolic inhibition (Fig. 5; see also Allen et al., 2011; Markelz et al., 2011). We included several experimental studies where the plants might have experienced metabolic inhibition (Samarakoon et al., 1995; Allen et al., 2011). Yet, if metabolic inhibition caused by low relative water content were the dominant stressor under drought for the majority of plants included in our analysis, we would not have

Fig. 5. Conceptual model of plant responses to elevated CO_2 under different drought intensities or phases during progressing drought. C_3 plants benefit from eCO_2 prior to drought. The dry period starts in phase I and leads to drought stress for plants grown under ambient CO_2 concentrations (not shown) but not for plants grown under eCO_2 , which exhibit reduced stomatal conductance. In phase II, lower stomatal conductance due to eCO_2 leads to small and large increases in biomass for C_3 and C_4 plants, respectively. In phase III, drought leads to low intercellular CO_2 concentrations (and a reduction in biomass) in C_3 , but not in C_4 plants. In phase IV a low intercellular CO_2 concentration limits both C_3 and C_4 plants; in phase V photosynthesis is fully limited by metabolic inhibition and an effect of eCO_2 is absent. Biomass under ambient CO_2 is set to zero. Red curve: C_3 plants; blue curve: C_4 plants. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

found the stimulating effect of eCO_2 , because metabolic inhibition can per definition not be alleviated by eCO_2 (Lawlor, 2002).

We summarized the duration of a theoretical experiment where plants were exposed to drought and eCO_2 in five phases (Fig. 5). In the first phase the plants do not experience drought stress and only C_3 plants experience growth stimulation by eCO_2 , as C_4 plants are (already) CO_2 saturated. In phase II, plants experience mild drought stress under ambient CO_2 , but exposure to eCO_2 leads to a delay of the onset of drought stress, because increased stomatal closure under eCO_2 has saved water. During this phase, the eCO_2 effect on growth of C_3 plants may be greater under drought. Phase III starts when (despite eCO_2) drought inhibits growth because further stomatal closure leads to large enough decreases in intercellular CO_2 to reduce carbon assimilation. In this phase biomass decreases for C_3 but not for C_4 plants, because C_4 plants are able to operate at a lower intercellular CO_2 -concentration and can therefore grow whilst exhibiting lower stomatal conductance. Consequently, the response in biomass of the two different metabolisms to eCO_2 becomes increasingly similar. During phase IV, low intercellular CO_2 concentrations limit both C_3 and C_4 plants due to very low stomatal conductance and incipient metabolic limitation, which leads to a similar response in biomass reduction to eCO_2 . The impact of metabolic inhibition increases when drought persists and the stimulating effect of eCO_2 continuously decreases, until in phase V photosynthesis is fully limited by metabolic inhibition and an effect of eCO_2 is absent. We suspect that most experimental data in the set analyzed in this paper result from plants harvested when the effect of drought stress has not (fully) diminished the effect of increased biomass accumulation under eCO_2 , i.e., phases III and IV in our theoretical experiment.

The results of this meta-analysis seem to contradict studies that showed stronger responses for C_3 crops to eCO_2 in dry, than in wet areas or seasons (Morgan et al., 2011; Bishop et al., 2014; Reyes-Fox et al., 2014). We suggest that this is due to the fundamental difference of experiments with experimentally imposed drought causing moderate to severe stress (as used in this analysis), and field studies with different amounts of precipitation causing mild to moderate drought stress (phases I and II in Fig. 5). In mild to moderate drought stress, the delay of drought stress effects due to

water savings and increased stomatal closure is of greater influence.

5. Conclusions

This meta-analysis of studies that experimentally superimposed water treatments over CO_2 exposure showed that crop biomass and yield respond similarly to eCO_2 . C_3 crops showed a consistent increase in both biomass and yield when grown under eCO_2 independent of the watering regime. In contrast, for C_4 crops the increase in yield and biomass was restricted to dry growing conditions. The results presented here suggest that under future CO_2 levels, C_4 crops will not forfeit their advantage over C_3 crops under drought conditions. This finding has important implications for presumed changes in C_3/C_4 species composition of ecosystems as well as the agricultural use of crops of both types and it furthermore supports attempts of breeding for higher drought tolerance of C_4 crops (Lopes et al., 2011; Mutava et al., 2011).

Author contributions

Study design: CJvdK and MT; Data collection: CJvdK and MR; Data analysis and interpretation: all authors. CJvdK and MR wrote the paper with input from all authors. All authors approved the article in its final form for submission. The authors declare responsibility of the integrity of the work as a whole.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.envexpbot.2015.10.004>.

References

- Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2 . *New Phytol.* 165, 351–372.
- Ainsworth, E.A., Rogers, A., 2007. The response of photosynthesis and stomatal conductance to rising $[\text{CO}_2]$: mechanisms and environmental interactions. *Plant Cell Environ.* 30, 258–270.
- Ainsworth, E.A., Leakey, A.D.B., Ort, D.R., Long, S.P., 2008. FACE-ing the facts: inconsistencies and interdependence among field: chamber and modeling studies of elevated $[\text{CO}_2]$ impacts on crop yield and food supply. *New Phytol.* 179, 5–9.
- Alfonso, S.U., Brüggemann, W., 2012. Photosynthetic responses of a C_3 and three C_4 species of the genus *Panicum* (sl) with different metabolic subtypes to drought stress. *Photosynthesis Res* 112, 175–191.
- Aljazairi, S., Arias, C., Nogués, S., 2015. Carbon and nitrogen allocation and partitioning in traditional and modern wheat genotypes under pre-industrial and future CO_2 conditions. *Plant Biol.* 17, 647–659.
- Allen, L.H., Kakani, V.G., Vu, J.C.V., Boote, K.J., 2011. Elevated CO_2 increases water use efficiency by sustaining photosynthesis of water-limited maize and sorghum. *J. Plant Physiol.* 168, 1909–1918.
- Bazzaz, F.A., 1990. The response of natural ecosystems to the rising global CO_2 levels. *Annu. Rev. Ecol. Syst.* 167–196.
- Bishop, K.A., Leakey, A.D.B., Ainsworth, E.A., 2014. How seasonal temperature or water inputs affect the relative response of C_3 crops to elevated $[\text{CO}_2]$: a global

- analysis of open top chamber and free air CO₂ enrichment studies. *Food Energy Secur.* 3, 33–45.
- Blum, A., 2005. Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? *Crop Pasture Sci.* 56, 1159–1168.
- Bowes, G., 1993. Facing the inevitable: plants and increasing atmospheric CO₂. *Ann. Rev. Pl. Biol.* 44, 309–332.
- Burkart, S., Manderscheid, R., Wittich, K.P., Löpmeier, F.J., Weigel, H.J., 2011. Elevated CO₂ effects on canopy and soil water flux parameters measured using a large chamber in crops grown with free-air CO₂ enrichment. *Plant Biol.* 13, 258–269.
- Campbell, D., Kondra, Z., 1978. Relationships among growth patterns, yield components and yield of rapeseed. *Can. J. Pl. Sci.* 58, 87–93.
- Cattivelli, L., Rizza, F., Badeck, F., Mazzucotelli, E., Mastrangelo, A.M., Francia, E., Mare, C., Tondelli, A., Stanca, A.M., 2008. Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. *Field Crops Res.* 105, 1–14.
- Champolivier, L., Merrien, A., 1996. Effects of water stress applied at different growth stages to *Brassica napus* L. var. *oleifera* on yield, yield components and seed quality. *Eur. J. Agron.* 5, 153–160.
- Cure, J.D., Acocck, B., 1986. Crop responses to carbon dioxide doubling: a literature survey. *Agric. for. Meteorol.* 38, 127–145.
- Curtis, P.S., Wang, X., 1998. A meta-analysis of elevated CO₂ effects on woody plant mass, form, and physiology. *Oecologia* 113, 299–313.
- Daynard, T., Tanner, J., Duncan, W., 1971. Duration of the grain filling period and its relation to grain yield in corn, *Zea mays* L. *Crop Sci.* 11, 45–48.
- Dias, M., Brüggemann, W., 2010. Water-use efficiency in *Flaveria* species under drought-stress conditions. *Photosynthetica* 48, 469–473.
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A., 2009. Plant drought stress: effects: mechanisms and management. *Agron. Sust. Dev.* 29, 153–188.
- Farquhar, G.D., von Caemmerer, S., Berry, J.A., 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* 149 (1), 78–90.
- Gebeyehou, G., Knott, D., Baker, R., 1982. Relations hips among durations of vegetative and grain filling phases, yield components, and grain yield in durum wheat cultivars. *Crop Sci.* 22, 287–290.
- Glass, G.V., 1976. Primary, secondary, and meta-analysis of research. *Edu. Res.* 5, 3–8.
- Grantz, D.A., Paudel, R., Vu, H.-B., Shrestha, A., Grulke, N., 2015. Diel trends in stomatal response to ozone and water deficit: a unique relationship of midday values to growth and allometry in Pima cotton? *Plant Biol.* doi: <http://dx.doi.org/10.1111/plb.12355> In press.
- Gupta, N., Gupta, S., Kumar, A., 2001. Effect of water stress on physiological attributes and their relationship with growth and yield of wheat cultivars at different stages. *J. Agron. Cr. Sci.* 186, 55–62.
- Hatch, M.D., 2002. C₄ photosynthesis: discovery and resolution. *Photosynth. Res.* 73, 251–256.
- Hawkesford, M.J., Araus, J., Park, R., Calderini, D., Miralles, D., Shen, T., Zhang, J., Parry, M.A., 2013. Prospects of doubling global wheat yields. *Food Energy Secur.* 2, 34–48.
- Hedges, L.V., Gurevitch, J., Curtis, P.S., 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* 80, 1150–1156.
- Higgins J., Green S., (2011). *Cochrane Handbook for Systematic Reviews of Interventions*. The Cochrane Collaboration.
- Högy, P., Wieser, H., Köhler, P., Schwadorf, K., Breuer, J., Franzaring, J., Muntiferer, R., Fangmeier, A., 2009. Effects of elevated CO₂ on grain yield and quality of wheat: results from a 3-year free-air CO₂ enrichment experiment. *Plant Biol.* 11, 60–69.
- Hovenden, M.J., Newton, P.C., Wills, K.E., 2014. Seasonal not annual rainfall determines grassland biomass response to carbon dioxide. *Nature* 511, 583–586.
- IPCC, 2014. *Climate Change 2014: Mitigation of Climate Change. Summary for Policymakers*. Cambridge University Press, United Kingdom.
- Jablonski, L.M., Wang, X., Curtis, P.S., 2002. Plant reproduction under elevated CO₂ conditions: a meta-analysis of reports on 79 crop and wild species. *New Phytol.* 156, 9–26.
- Jenner, C., Ugalde, T., Aspinall, D., 1991. The physiology of starch and protein deposition in the endosperm of wheat. *Funct. Pl. Biol.* 18, 211–226.
- Kadam, N.T., Xiao, G., Jean Melgar, R., Bahuguna, R.N., Quinones, C., Tamilselvan, A., Prasad, P.V.V., Jagadish, K.S.V., 2014. Agronomic and physiological responses to high temperature drought: and elevated CO₂ in cereals. *Adv. Agron.* 127, 111–156.
- Kimball, B.A., 1983. Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agron. J.* 75, 779–788.
- Kirkham, M.B., He, H., Bolger, T.P., Lawlor, D.J., Kanemasu, E.T., 1991. Leaf photosynthesis and water use of big bluestem under elevated carbon dioxide. *Crop Sci.* 31, 1589–1594.
- Kirkham, M.B., 2011. *Elevated Carbon Dioxide: Impacts on Soil and Plant Water Relations*. CRC Press, Boca Raton, pp. 416.
- Lawlor, D.W., 2002. Limitation to photosynthesis in water-stressed leaves: stomata vs. metabolism and the role of ATP. *Ann. Bot.* 89, 871–885.
- Lawlor, D.W., Mitchell, R.A.C., 1991. The effects of increasing CO₂ on crop photosynthesis and productivity: a review of field studies. *Plant Cell Environ.* 14, 807–818.
- Leakey, A.D., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P., Ort, D.R., 2009. Elevated CO₂ effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *J. Exp. Bot.* 60, 2859–2876.
- Leakey, A.D., Uribealraa, M., Ainsworth, E.A., Naidu, S.L., Rogers, A., Ort, D.R., Long, S.P., 2006. Photosynthesis productivity, and yield of maize are not affected by open-air elevation of CO₂ concentration in the absence of drought. *Plant Physiol.* 140, 779–790.
- LeCain, D.R., Morgan, J.A., Mosier, A.R., Nelson, J.A., 2003. Soil and plant water relations determine photosynthetic responses of C₃ and C₄ grasses in a semi-arid ecosystem under elevated CO₂. *Ann. Bot.* 92, 41–52.
- Li, Y., Ye, W., Wang, M., Yan, X., 2009. Climate change and drought: a risk assessment of crop-yield impacts. *Clim. Res.* 39, 31.
- Lobell, D.B., Roberts, M.J., Schlenker, W., Braun, N., Little, B.B., Rejesus, R.M., Hammer, G.L., 2014. Greater sensitivity to drought accompanies maize yield increase in the US Midwest. *Science* 344, 516–519.
- Lopes, M.S., Araus, J.L., van Heerden, P.D., Foyer, C.H., 2011. Enhancing drought tolerance in C₄ crops. *J. Exp. Bot.* 62, 3135–3153.
- Manderscheid, R., Erbs, M., Weigel, H., 2014. Interactive effects of free-air CO₂ enrichment and drought stress on maize growth. *Eur. J. Agron.* 52, 11–21.
- Markelz, R.J., Strellner, R.S., Leakey, A.D., 2011. Impairment of C₄ photosynthesis by drought is exacerbated by limiting nitrogen and ameliorated by elevated [CO₂] in maize. *J. Exp. Bot.* 62, 3235–3246.
- Miranda-Apodaca, J., Pérez-López, U., Lacuesta, M., Mena-Petite, A., Muñoz-Rueda, A., 2015. The type of competition modulates the ecophysiological response of grassland species to elevated CO₂ and drought. *Plant Biol.* 17, 298–310.
- Morgan, J., Pataki, D., Körner, C., Clark, H., Del Grosso, S., Grünzweig, J., Knapp, A., Mosier, A., Newton, P., Niklaus, P., 2004. Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO₂. *Oecologia* 140, 11–25.
- Morgan, J.A., LeCain, D.R., Pendall, E., Blumenthal, D.M., Kimball, B.A., Carrillo, Y., Williams, D.G., Heisler-White, J., Dijkstra, F.A., West, M., 2011. C₄ grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature* 476, 202–205.
- Morison, J., Gifford, R., 1984. Plant growth and water use with limited water supply in high CO₂ concentrations. I. Leaf area, water use and transpiration. *Funct. Pl. Biol.* 11, 361–374.
- Murphy, K., Dawson, J., Jones, S., 2008. Relationship among phenotypic growth traits, yield and weed suppression in spring wheat landraces and modern cultivars. *Field Crops Res.* 105, 107–115.
- Mutava, R., Prasad, P., Tuinstra, M., Kofoed, K., Yu, J., 2011. Characterization of sorghum genotypes for traits related to drought tolerance. *Field Crops Res.* 123, 10–18.
- Nakagawa, S., Cuthill, I.C., 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol. Rev.* 82, 591–605.
- Nass, H., Reiser, B., 1975. Grain filling period and grain yield relationships in spring wheat. *Can. J. Pl. Sci.* 55, 673–678.
- Nelson, J.A., Morgan, J.A., LeCain, D.R., Mosier, A.R., Milchunas, D.G., Parton, B.A., 2004. Elevated CO₂ increases soil moisture and enhances plant water relations in a long-term field study in semi-arid shortgrass steppe of Colorado. *Plant Soil* 259, 169–179.
- Nemani, R.R., Keeling, C.D., Hashimoto, H., Jolly, W.M., Piper, S.C., Tucker, C.J., Myneni, R.B., Running, S.W., 2003. Climate-driven increases in global terrestrial net primary production from 1982 to 1999. *Science* 300, 1560–1563.
- Niklaus, P.A., Spillner, D., Körner, C., 1998. Soil moisture dynamics of calcareous grassland under elevated CO₂. *Oecologia* 117, 201–208.
- Norby, R.J., Zak, D.R., 2011. Ecological lessons from free-air CO₂ enrichment (FACE) experiments. *Ann. Rev. Ecol. Evol. Syst.* 42, 181.
- Pearcy, R., Ehleringer, J., 1984. Comparative ecophysiology of C₃ and C₄ plants. *Plant. Cell Environ.* 7, 1–13.
- Peterson, P., Sheaffer, C., Hall, M., 1992. Drought effects on perennial forage legume yield and quality. *Agron. J.* 84, 774–779.
- Poorter, H., 1998. Do slow-growing species and nutrient-stressed plants respond relatively strongly to elevated CO₂? *Global Change Biol.* 4, 693–697.
- Poorter, H., 1993. Interspecific variation in the growth response of plants to an elevated ambient CO₂ concentration. *Vegetatio* 104, 77–97.
- Poorter, H., Navas, M., 2003. Plant growth and competition at elevated CO₂: on winners: losers and functional groups. *New Phytol.* 157, 175–198.
- Core Team, R., 2012. *R: A Language and Environment for Statistical Computing*. The R Foundation for Statistical Computing, Vienna.
- Rampino, P., Pataleo, S., Gerardi, C., Mita, G., Perrotta, C., 2006. Drought stress response in wheat: physiological and molecular analysis of resistant and sensitive genotypes. *Plant Cell Environ.* 29, 2143–2152.
- Reich, M., van den Meerakker, A., Parmar Patel, A., Hawkesford, M.J., De Kok, L.J., 2015. Temperature and nitrogen form determine size and direction of effects of elevated CO₂ on quantity and quality of yield in Chinese cabbage. *Plant Biol.* doi: <http://dx.doi.org/10.1111/plb.12396> In press.
- Reyes-Fox, M., Steltzer, H., Trlica, M., McMaster, G.S., Andales, A.A., LeCain, D.R., Morgan, J.A., 2014. Elevated CO₂ further lengthens growing season under warming conditions. *Nature* 510, 259–262.
- Sadras, V.O., 2009. Does partial root-zone drying improve irrigation water productivity in the field? A meta-analysis. *Irrig. Sci.* 27, 183–190.
- Sage, R.F., Wedin, D.A., Li, M., 1999. The biogeography of C₄ photosynthesis: patterns and controlling factors. *C4 Plant Biol.* 313–373.
- Samarakoon, A., Muller, W., Gifford, R., 1995. Transpiration and leaf area under elevated CO₂: effects of soil water status and genotype in wheat. *Funct. Plant Biol.* 22, 33–44.
- Singh, S., Singh, G., Singh, P., Singh, N., 2008. Effect of water stress at different stages of grain development on the characteristics of starch and protein of different wheat varieties. *Food Chem.* 108, 130–139.
- Taeger, S., Sparks, T.H., Menzel, A., 2015. Effects of temperature and drought manipulations on seedlings of Scots pine provenances. *Plant Biol.* 17, 361–372.
- Tausz-Posch, S., Norton, R.M., Seneweera, S., Fitzgerald, G.J., Tausz, M., 2013. Will intra-specific differences in transpiration efficiency in wheat be maintained in a high CO₂ world? A face study. *Physiol. Plant.* 148, 232–245.

- Tollenaar, M., Lee, E., 2006. Dissection of physiological processes underlying grain yield in maize by examining genetic improvement and heterosis. *Maydica* 51, 399.
- Tyree, M.T., Alexander, J.D., 1993. Plant water relations and the effects of elevated CO₂: a review and suggestions for future research. *Vegetatio* 104, 47–62.
- Vandeger, R., Miller, R.E., Bain, M., Gleadow, R.M., Cavagnaro, T.R., 2013. Drought adversely affects tuber development and nutritional quality of the staple crop cassava (*Manihot esculenta* Crantz). *Funct. Plant Biol.* 40, 195–200.
- Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. *J. Stat. Software* 36, 1–48.
- Volk, M., Niklaus, P.A., Körner, C., 2000. Soil moisture effects determine CO₂ responses of grassland species. *Oecologia* 125, 380–388.
- Wall, G.W., Brooks, T.J., Adam, N.R., Cousins, A.B., Kimball, B.A., Pinter, P.J., LaMorte, R.L., Triggs, J., Ottman, M.J., Leavitt, S.W., Matthias, A.D., Williams, D.G., Webber, A.N., 2001. Elevated atmospheric CO₂ improved *Sorghum* plant water status by ameliorating the adverse effects of drought. *New Phytol.* 152, 231–248.
- Wall, G.W., 2001. Elevated atmospheric CO₂ alleviates drought stress in wheat. *Agric. Ecosyst. Environ.* 87, 261–271.
- Ward, J.K., Tissue, D.T., Thomas, R.B., Strain, B.R., 1999. Comparative responses of model C₃ and C₄ plants to drought in low and elevated CO₂. *Global Change Biol.* 5, 857–867.
- Wujeska, A., Bossinger, G., Tausz, M., 2013. Responses of foliar antioxidative and photoprotective defence systems of trees to drought: a meta-analysis. *Tree Physiol.* 33, 1018–1029.
- Wujeska-Klaue, A., Bossinger, G., Tausz, M., 2015. Seedlings of two Acacia species from contrasting habitats show different photoprotective and antioxidative responses to drought and heatwaves. *Ann. For. Sci.* 72, 403–414.
- Wullschlegel, S., Tschaplinski, T., Norby, R., 2002. Plant water relations at elevated CO₂—implications for water-limited environments. *Plant Cell Environ.* 25, 319–331.
- Yoshida, S., 1972. Physiological aspects of grain yield. *Ann. Pl. Phys.* 23, 437–464.
- Zelitch, I., 1982. The close relationship between net photosynthesis and crop yield. *Bioscience* 32, 796–802.
- Ziska, L.H., Namuco, O., Moya, T., Quilang, J., 1997. Growth and yield response of field-grown tropical rice to increasing carbon dioxide and air temperature. *Agron. J.* 89, 45–53.