

Second generation bioenergy crops and climate change: a review of the effects of elevated atmospheric CO₂ and drought on water use and the implications for yield

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Abstract

Second-generation, dedicated lignocellulosic crops for bioenergy are being hailed as the sustainable alternative to food crops for the generation of liquid transport fuels, contributing to climate change mitigation and increased energy security. Across temperate regions they include tree species grown as short rotation coppice and intensive forestry (e.g. *Populus* and *Salix* species) and C₄ grasses such as miscanthus and switchgrass. For bioenergy crops it is paramount that high energy yields are maintained in order to drive the industry to an economic threshold where it has competitive advantage over conventional fossil fuel alternatives. Therefore, in the face of increased planting of these species, globally, there is a pressing need for insight into their responses to predicted changes in climate to ensure these crops are 'climate proofed' in breeding and improvement programmes. In this review, we investigate the physiological responses of bioenergy crops to rising atmospheric CO₂ ([Ca]) and drought, with particular emphasis on the C₃ *Salicaceae* trees and C₄ grasses. We show that while crop yield is predicted to rise by up to 40% in elevated [Ca], this is tempered by the effects of water deficit. In response to elevated [Ca] stomatal conductance and evapotranspiration decline and higher leaf–water potentials are observed. However, whole-plant responses to [Ca] are often of lower magnitude and may even be positive (increased water use in elevated [Ca]). We conclude that rising [Ca] is likely to improve drought tolerance of bioenergy crop species due to improved plant water use, consequently yields in temperate environments may remain high in future climate scenarios.

Keywords: bioenergy crop, climate change, drought, elevated CO₂, water stress, water use

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Introduction

With atmospheric CO₂ [Ca] concentrations predicted to rise steadily and significantly (IPCC, 2007), the increased exploitation of renewable energy sources is likely to make an increasing contribution to climate change mitigation and also improve energy security. One source of renewable energy that has much to offer, being both sustainable and relatively carbon neutral, is biomass from dedicated bioenergy crops.

Predicted consequences of continued increases in [Ca] and other greenhouse-gas emissions include changes in the intensity and frequency of climate

extremes such as heat waves, heavy precipitation and drought. In the higher latitudes of the Northern Hemisphere, changes in rainfall are predicted by general circulation model (GCM) simulations (IPCC, 2001). In the United Kingdom, for example, warmer, wetter winters and drier summers are predicted, with an increase in the variability and intensity of rainfall and drought (Hulme *et al.*, 2002), while in the United States, the frequency of drought is predicted to increase in the west (Bates *et al.*, 2008). In general, more frequent and prolonged summer droughts appear likely across northern latitudes. Increased carbon uptake by plants in response to rising concentrations of [Ca], termed the 'CO₂ fertilization effect', can create a negative feedback slowing the rate of increase in [Ca], consequently slowing the rate of change in climate predicted from

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increasing [Ca] concentrations. It is also predicted to generate higher plant biomass (Norby *et al.*, 2005). However, this response is variable, depending on species and plant age (Körner *et al.*, 2005; Körner, 2006) and is likely to be tempered by interaction with other environmental stresses such as water deficit and temperature (Oren *et al.*, 2001). Investigation of elevated [Ca] in concert with stresses such as nutrient availability and atmospheric ozone concentration have already been shown to modify the CO₂ response of some plant species (Oren *et al.*, 2001; Karnosky *et al.*, 2003). Therefore, it is important to understand plant responses to 'multiple' climate changes, not just elevated [Ca].

In this review, we investigate the physiological responses of second-generation lignocellulosic bioenergy crops to the concurrent climate effects of elevated [Ca] and reduced summer precipitation. We focus on dedicated second-generation lignocellulosic bioenergy crops – those crops grown solely for the purpose of energy production from their woody and fibrous cell wall biomass, i.e. with no role in food production. In temperate latitudes, these are mainly perennial species including C₃ woody crops of willow (*Salix* spp.) and poplar (*Populus* spp.), grown under short rotation coppice (SRC) or intensive single stem forest management, and C₄ grasses such as miscanthus (*Miscanthus giganteus*) and switchgrass (*Panicum virgatum*). In tropical and subtropical areas, the oil crop *Jatropha* (*Jatropha curcas*) has been suggested as a dedicated second generation bioenergy crop (Openshaw, 2000). Sugar cane, although currently grown as a fuel crop utilizing sugar, may in future also be grown for fibrous stems and leaves. Elevated [Ca] and drought affect both plant productivity and water use. Enhanced rates of photosynthesis and reduced stomatal conductance (*g_s*) are well documented responses of growth in elevated [Ca] which increase plant water use efficiency (WUE) i.e. the amount of biomass produced per unit water used (Ceulemans & Mousseau, 1994; Gunderson & Wulfschleger, 1994; Drake *et al.*, 1997; Curtis & Wang, 1998; Saxe *et al.*, 1998; Norby *et al.*, 1999). It is suggested that these responses to an enriched CO₂ atmosphere will improve the drought tolerance of plants by delaying the onset of drought due to enhanced soil water availability. Although the effects of elevated [Ca] on plant functioning have been addressed in many reviews (Ceulemans & Mousseau, 1994; Drake *et al.*, 1997; Saxe *et al.*, 1998; Medlyn *et al.*, 1999; Long *et al.*, 2004; Ainsworth & Long, 2005; Ainsworth & Rogers, 2007), uniquely in this review we focus on the combined effects of elevated [Ca] and drought and the implications this has for bioenergy crop production. The importance of adequate water availability for plant growth and ecosystem functioning cannot be overstated. Currently, low water availability

is the main factor limiting plant growth and yield worldwide (Chaves *et al.*, 2003) and, as suggested by GCM predictions, global change will make water scarcity ever more prominent in many parts of the world. Drought is therefore likely to be the change in climate that has the greatest impact on plant growth, productivity and ecosystem function in most parts of the temperate latitudes, so much so that its effect may well mask any effect of elevated [Ca]. Therefore, we propose that while growth in an elevated CO₂ atmosphere may have beneficial effects on bioenergy crop productivity and water use, this will be strongly dependent on the severity, timing and frequency of drought during the growing season, which could ultimately off-set any positive effects of plant growth in an elevated [Ca] environment.

The implications of climate change for bioenergy crop production are substantial. For the industry to compete successfully with conventional fossil fuel alternatives and flourish, energy yield per hectare must be high and needs to remain so in the face of a changing climate. Crop breeding and improvement programs provide a means to do this and the complete sequencing of the poplar genome suggests significant advances are being made (Tuskan *et al.*, 2006). Decoupling yield from water use is recognized as a difficult breeding target although recent progress with wheat in Australia suggests that this is possible (Condon *et al.*, 2004; Pennisi, 2008; Finkel, 2009). Unlike bioenergy crops such as sugar cane and maize that have been grown in intensive management systems for decades and subjected to years of plant improvement, the C₃ *Salicaceae* trees and C₄ grasses under focus here are different in that they are relatively unimproved by breeding programs, and are grown in managed 'cropping' systems which are unusual for these species. Another concern about bioenergy crop production in a future climate surrounds environmental impacts of bioenergy crop growth particularly in relation to reduced water availability. The C₃ *Salicaceae* trees in particular have high rates of water use (Lindroth *et al.*, 1994; Lindroth & Cienciala, 1996; Hall *et al.*, 1998) which, should droughts become more frequent as is forecast, pose a serious threat to ecosystem water resources. More than ever there is a need to quantify and predict the effects of increasing [Ca] and drought on crop growth and ecosystem functioning specifically for these species for both economic and environmental reasons.

Information specific to perennial bioenergy crops that are the focus of this review is limited; therefore we draw on examples from other C₃ tree species and C₄ grass species where necessary. The development in recent years of large-scale free-air CO₂ enrichment (FACE) experiments, allowing the exposure of plants to elevated [Ca] under natural, field conditions, has

contributed significantly to our understanding of plant responses to rising [Ca] and a recent synthesis of outputs from these experiments suggests that yield enhancement in elevated [Ca] is likely to be less than that predicted from work in small- and open-top chamber (OTC) studies (Long *et al.*, 2006). Well known limitations exist to the use of controlled environment and OTC systems when studying plant physiological responses to elevated [Ca] (Arp, 1991; Long *et al.*, 2004; Nowak *et al.*, 2004), consequently our synthesis is based mainly on FACE experiments. Globally, there are many FACE experiments of which two, AspenFACE (Rhinelander, WI, USA), and popFACE (Viterbo, Central Italy), use varieties of poplar. *Populus tremuloides* is grown at AspenFACE, and *P. alba*, *P. × euramericana* and *P. nigra* are grown at popFACE under SRC management. Data for willow are extremely limited; therefore we draw upon examples from other C_3 woody temperate species where necessary. Data for C_4 bioenergy crops, such as miscanthus and switchgrass, are virtually nonexistent, highlighting a gap in research. Examples are drawn from other C_4 grasses and agricultural crops including sorghum (*Sorghum bicolor*) and maize (*Zea mays*) among others.

Rates of water use of second-generation bioenergy crops in the current climate

Although plant water use is dependent on genotypic variation, climate and soil conditions, many studies have demonstrated particularly high water use for second-generation lignocellulosic bioenergy crops. An early study of the water use of irrigated poplar coppice carried out in Wisconsin, USA by Hansen (1988) found maximum stand transpiration rates of 4.4–4.8 mm per day during the second to fifth growing seasons. Hinckley *et al.* (1994) found similar maximum stand transpiration rates of 4.8 mm per day for a 4-year-old uncoppiced stand of *Populus trichocarpa* × *Populus deltoides*. Field measurements of transpiration rates from two poplar genotypes (Beaupré and Dorschkamp) at sites in the United Kingdom revealed very high transpiration rates, typically up to 8 mm per day during the growing season (Hall & Allen, 1997). Similarly high transpiration rates were estimated for willow at the same site (Hall *et al.*, 1998). Studies on irrigated willow SRC in Sweden (Lindroth *et al.*, 1994; Lindroth & Cienciala, 1996) and varieties of poplar SRC in the United Kingdom (Hall *et al.*, 1996) have shown that they use significantly more water than agricultural crops and most broadleaved tree species, but not conifers. Less information is available about the water use of miscanthus or switchgrass, but a report by Finch *et al.* (2004), which used a combination of measurements and modelling to quantify

evaporation, concluded that although the transpiration rates from miscanthus were high during the growing season, on an annual basis they were comparable to permanent grassland. Nevertheless, such seasonal heavy water use demonstrated by miscanthus in synchrony with summer drought could lead to higher impacts on ecosystem water availability compared with permanent grassland.

Plant responses to elevated atmospheric CO₂

In an attempt to simplify the plant physiological responses to elevated [Ca] for C_3 and C_4 lignocellulosic bioenergy crops, we identified two pathways, 'direct' and 'indirect', through which elevated [Ca] affects photosynthesis (Fig. 1). The 'direct' CO₂ effect describes the pathway through which elevated [Ca] concentration directly influences net CO₂ assimilation by altering photosynthetic metabolic processes. The 'indirect' CO₂ pathway describes how elevated [Ca] can influence net photosynthesis indirectly via stomatal regulation. An increase in net carbon assimilation rate as a result of either pathway leads to increased capacity to generate energy for growth, ultimately resulting in higher biomass production, which is desirable for a bioenergy crop species (Ceulemans & Mousseau, 1994; Curtis & Wang, 1998; Norby *et al.*, 1999; Gielen & Ceulemans, 2001; Calfapietra *et al.*, 2003; Poorter & Navas, 2003; Ainsworth & Long, 2005; Liberloo *et al.*, 2006), although this plant response is variable (Körner *et al.*, 2005). It is beyond the scope of this review to investigate the allocation of increased carbon production within plants as a result of elevated [Ca], and the mechanism through which this occurs via the 'direct' pathway. However, although not addressed in detail in this review, this is also an area of significant interest for bioenergy crop species where maximum allocation of carbon to above-ground structural carbon pools (e.g. cellulose, hemicellulose and lignin) would be advantageous to increase yield (Luo *et al.*, 2006). Instead, this review concentrates on the 'indirect' pathway. While we recognize that the 'direct' CO₂ effect is a necessary component of increased plant WUE, it is not the dominant pathway through which plant water relations are controlled, which is the focus of this review. For further details of plant photosynthetic responses to elevated [Ca] readers are directed to the reviews of (Curtis & Wang, 1998; Saxe *et al.*, 1998; Medlyn *et al.*, 1999; Wand *et al.*, 1999; Nowak *et al.*, 2004; Ainsworth & Long, 2005).

The stomatal response of C_3 and C_4 plants

It has been observed that elevated [Ca] has significant positive effects on plant water relations in both C_3 and

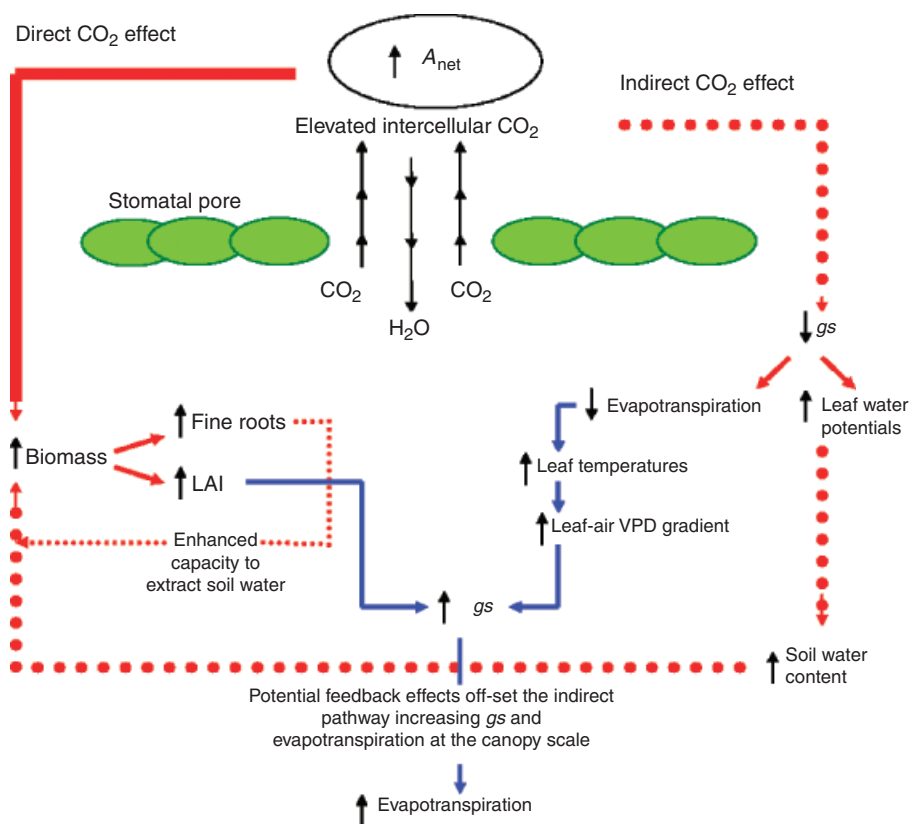


Fig. 1 The main pathways through which elevated atmospheric CO₂ affects plant biomass and water use. ■ Denotes the pathway through which elevated CO₂ directly stimulates photosynthetic metabolism, increasing net carbon assimilation (A_{net}) and thus biomass. The direct stimulation of photosynthesis in C₃ plants is well documented and mechanisms understood. In C₄ plants direct stimulation of photosynthesis is unclear, it has been observed in some studies, but proposed mechanisms are not well understood and theory suggests C₄ species would not respond to elevated CO₂.

● ● Denotes the pathway through which elevated CO₂ has an indirect effect on plant photosynthesis, thus increasing biomass. Decreased stomatal conductance (g_s) leads to improved plant–water relations. Transpirational water loss is reduced, which results in the maintenance of higher leaf water potentials and higher soil water content over the course of the growing season. Together these ameliorate the negative effects of drought by delaying the development of water stress in the plant which may inhibit plant photosynthesis and reduce growth.

— Denotes potential negative feedbacks that may off-set the beneficial effects of elevated CO₂ on plant water use. Increased leaf area index (LAI) may result in higher leaf g_s , thus evapotranspiration at the canopy scale will increase. Another potential scenario suggests decreased evapotranspiration from reduced g_s may lead to higher leaf temperatures. This may decrease humidity within the canopy and thus increase the leaf-to-air vapour pressure gradient, ultimately increasing the driving force for transpiration within the canopy.

C₄ plants as a consequence of decreased stomatal conductance (g_s) (Drake *et al.*, 1997; Wand *et al.*, 1999; Lee *et al.*, 2001; Medlyn *et al.*, 2001; Gunderson *et al.*, 2002; Ainsworth *et al.*, 2003; Ainsworth & Long, 2005; Ainsworth & Rogers, 2007). Water loss and CO₂ uptake in higher plants is tightly regulated by stomata present on the leaf epidermis (for a detailed review of stomatal pore functioning see Vavasseur & Raghavendra, 2005). Stomatal pores allow plants to dynamically control carbon assimilation and water balance, which ultimately leads to a compromise between the photosynthetic requirement for CO₂ and the availability of water. Due to the control exerted by stomata over

transpirational water loss and net CO₂ assimilation, quantifying stomatal responses to rising [Ca] concentrations are vital in order to determine the effects of climate change on plant growth and biomass accumulation, and the consequence of this on water availability within ecosystems. In addition, plant transpiration comprises a major regulator of mass and energy exchange between vegetation and the atmosphere (Garratt, 1992). Understanding the long-term impacts of climate change on plant water use is critical as, at regional and continental scales, this will feedback to impact atmospheric conditions. As significant amounts of water evaporated from land masses pass through

Table 1 Average change (%) in stomatal conductance (g_s) with elevated atmospheric CO_2 for a range of C_3 woody species, C_3 and C_4 grasses

| Species | Average change in g_s with elevated CO_2 | Experiment | Length of study | Reference |
|--|--|-----------------------|------------------|------------------------------------|
| <i>C₃ woody species:</i> | | | | |
| <i>Populus x euramericana</i> (genotype I-214, poplar) | ↓ 19% (low N) ↓ 24% (high N) | FACE | 5 years | Tricker <i>et al.</i> (2005) |
| <i>Liquidambar styraciflua</i> L. (Sweetgum) | ↓ 24% (upper canopy) ↓ 14% (mid canopy) | FACE | 3 years | Gunderson <i>et al.</i> (2002) |
| <i>Liquidambar styraciflua</i> L. (Sweetgum) | ↓ 31% (upper canopy) ↓ 25% (mid canopy) | FACE | 4 years | Herrick <i>et al.</i> (2004) |
| <i>Liquidambar styraciflua</i> L. (Sweetgum) | ↓ 22/23% | FACE | 1 growing season | Wullschlegel <i>et al.</i> (2002a) |
| <i>Larrea tridentata</i> (L. divaricata) (creosote bush) | ↓ 13% | FACE | 3 years | Nowak <i>et al.</i> (2004) |
| <i>Populus tremuloides</i> Michx. | ↓ | FACE + O ₃ | 1 year | Noormets <i>et al.</i> (2001) |
| <i>C₃ grasses:</i> | | | | |
| <i>Poaceae</i> (various, meta-analysis) (grasses) | ↓ 24% | FACE, OTC & CE | n/a | Wand <i>et al.</i> (1999) |
| <i>Triticum aestivum</i> (L. cv. 'Yecora Rojo') (spring wheat) | ↓ 36% | FACE | 1 growing season | Garcia <i>et al.</i> (1998) |
| <i>Achnatherum hymenoides</i> (Roemer & Schultes) (Indian ricegrass) | ↓ 20% | FACE | 3 years | Nowak <i>et al.</i> (2004) |
| <i>C₄ grasses:</i> | | | | |
| <i>Poaceae</i> (various, meta-analysis) (grasses) | ↓ 29% | FACE, OTC & CE | n/a | Wand <i>et al.</i> (1999) |
| <i>Zea mays</i> (maize) | ↓ 34% | FACE | 1 growing season | Leakey <i>et al.</i> (2006) |
| <i>Sorghum bicolor</i> (sorghum) | ↓ 32% (limited soil water) ↓ 37% (ample soil water) | FACE | 1 growing season | Wall <i>et al.</i> (2001) |
| <i>Lolium perenne</i> (L. cv. Bastion) (perennial ryegrass) | ↓ 30% | FACE | 10 years | Ainsworth <i>et al.</i> (2003) |
| <i>Pleuraphis rigida</i> (Thurber) (big galleta grass) | ↓ 35% | FACE | 3 years | Nowak <i>et al.</i> (2004) |

Results were taken from the literature reported as the average of all observations, for more details refer to individual references. ↓, decreased g_s ; N, nitrogen; FACE, free-air CO_2 enrichment; O₃, ozone; OTC, open top chamber; CE, controlled environment.

stomata, the effect of climate changes such as drought and elevated [Ca] will have substantial impact on the water balance and vegetation–climate feedbacks, and consequently future climate conditions (Körner *et al.*, 2007).

Intercellular CO_2 concentration is a key variable sensed by guard cells and used to co-ordinate stomatal opening (Tricker *et al.*, 2005). The stomatal response to changes in [Ca] concentration, however, is also tightly dependent on many other variables such as light intensity, plant water status, temperature and atmospheric vapour pressure deficit (VPD). These are all key abiotic factors likely to change as a result of rising [Ca] concentrations.

Table 1 summarizes changes in g_s observed under elevated [Ca] for a range of C_3 woody species and C_3 and C_4 grasses. Interestingly, varieties of poplar growing under SRC management at the popFACE facility in Italy were found to change their stomatal response to

elevated [Ca] depending on age. Precoppice stages of all three genotypes measured (*P. x euramericana*, *P. alba* and *P. nigra*) showed no g_s response with FACE treatment (Bernacchi *et al.*, 2003). Following coppicing however, during the growing season, a significant decrease in stomatal response was observed only in *P. nigra*. In a mature closed-canopy bioenergy plantation, g_s measured in *P. x euramericana* alone decreased in response to FACE treatment in combination with soil nitrogen concentration, a 19% decrease at low nitrogen concentration and 24% decrease at high nitrogen concentration (Tricker *et al.*, 2005). This long-term study of different genotypes over different life cycle stages highlights the difficulty of extrapolating and predicting plant or ecosystem responses to elevated [Ca] and also raises awareness of the potential impacts of management regime on plant responses to environmental change.

There are no FACE experimental results for the tree genus *Salix* or for the C₄ grasses switchgrass and miscanthus. The response of willow, however, is likely to be similar to the SRC poplar, both being managed by the same SRC system and sharing similar characteristics such as fast growth rates, high biomass accumulation and high water use.

A meta-analysis of species grown in elevated CO₂ at different FACE facilities showed that *g_s* decreased on average by 20% in elevated [Ca], and there was no difference in the response between C₃ and C₄ species (Ainsworth & Long, 2005). This result summarizing plant functional group responses to elevated [Ca] is interesting and suggests that when grown in realistic field conditions, no differences in *g_s* are apparent between C₃ trees and C₄ grasses in response to [Ca]. Currently plants using the C₄ photosynthetic pathway are generally considered more water use efficient as the C₄ pathway evolved as an adaptation to arid conditions, being most efficient under high light intensity and high temperatures. Assumptions based on photosynthetic theory suggest that C₄ species would not benefit from increases in [Ca] because of the 'CO₂ concentrating mechanism in the bundle sheath cells', i.e. C₄ species are substrate saturated at current [Ca] levels (Wand *et al.*, 1999). Conversely, as C₃ plants are substrate limited at current ambient CO₂ levels, a strong stimulation of net photosynthesis is predicted with increasing [Ca]. Downregulation over time of photosynthetic capacity in elevated [Ca] has been observed in some species (Huxman *et al.*, 1998; Rogers & Ellsworth, 2002; Ainsworth *et al.*, 2003), but not in others (Tognetti *et al.*, 1999; Herrick & Thomas, 2001; Gunderson *et al.*, 2002). Current work suggests limited nitrogen supply and a reduced capacity to utilize photosynthate (sink strength) may explain these responses (Moore *et al.*, 1998, 1999; Stitt & Krapp, 1999; Long *et al.*, 2004; Ainsworth & Rogers, 2007). For *Populus* species growing under SRC management in a bioenergy plantation, however, long-term downregulation of photosynthesis was avoided because the trees displayed a large photosynthate sink capacity (Davey *et al.*, 2006), suggesting these genotypes will be well suited for growth in a future climate being able to take advantage of elevated [Ca] concentrations and increase productivity.

Thus it may be that under future climate scenarios, C₃ *Salicaceae* trees will be favoured as a bioenergy crop for wide-scale planting over C₄ grasses as they display higher WUE and are able to maintain higher photosynthetic rates. Due to the longevity of perennial bioenergy crop plantations [approximately 25 year anticipated for SRC and 20 year for miscanthus; (Karp & Shield, 2008)] foresight of suitable species to plant with reference to future climate conditions is necessary to prevent poten-

tial economic losses. Nevertheless, the variable stomatal response to elevated [Ca] observed in different *Populus* genotypes at varying stages in the SRC growth cycle raises concerns about water use of these species in a future enriched CO₂ atmosphere.

The mechanism of reduced stomatal conductance in elevated atmospheric CO₂

The mechanism by which *g_s* is reduced in elevated [Ca] is complex. Original research suggested the observed decline in *g_s* was due to a decline in stomatal density or frequency (Woodward, 1987). Stomatal density determines the maximum *g_s* that a unit area of leaf can attain; therefore an acclamatory response via change in stomatal numbers is often suggested. Historical evidence across geological time from herbarium and fossil records suggests a decrease in stomatal density in trees with increasing [Ca] (Woodward, 1987; Van der Burgh *et al.*, 1993), but this can only ever be correlative rather than causative. Beerling *et al.* (1996) analysed stomatal density of leaves grown in ambient and elevated [Ca] covering the period AD 1800–1994, which represents a change in CO₂ from 283–350 ppm. The three species studied, (Beech: *Fagus sylvatica* L.; Birch: *Betula pubescens* Ehrh.; and Oak: *Quercus robur* L.), showed a decrease in stomatal density, with the steepest decline between 1927 and 1994. Woodward (1987) attributed a 40% decrease in stomatal density in the leaves of herbarium samples of tree species collected over the last 200 years to the increase in [Ca]. Poole *et al.* (1996), however, carried out an intense survey of stomatal density from leaves of *Alnus glutinosa* from over 70 sites. They found that stomatal density differed, with considerable variation from measurements within the same tree. This decreases the reliability of historical results of those such as Beerling *et al.* (1996) and Woodward (1987) and also highlights another limitation of historical studies since in the majority only stomatal density and not stomatal index was measured. Stomatal density is the number of stomata in a given area of epidermis and can be affected indirectly by the expansion of epidermal cells, which are also known to be sensitive to [Ca] (Taylor *et al.*, 2003). Stomatal index, in contrast, is the density of stomata expressed as a percentage of the density of epidermal cells plus stomata (Poole *et al.*, 1996).

Manipulative studies using elevated [Ca] also give variable results. Radoglou & Jarvis (1990b) showed that growth of four hybrid poplars at elevated [Ca] did not affect stomatal density, index or length of stomatal pore. A meta-analysis of stomatal density responses to elevated CO₂ across FACE studies by Ainsworth & Rogers (2007) found an average 5% decrease in density,

but this was not statistically significant. Because stomatal density is a feature that is established during the early stages of leaf development, Radoglou & Jarvis (1990) suggested that elevated CO_2 has no direct effects on the initiation of the number of stomata during ontogenesis, or on epidermal cell expansion at a later stage, instead g_s decreases because of the effect of CO_2 on stomatal opening. In support of this Tricker *et al.* (2005) showed that after five growing seasons the g_s of *P. × euramericana* still responded to FACE treatment, whereas the frequency of stomata (density and index), which had decreased during the first 2 years of exposure to elevated [Ca], did not. This suggests that changes in stomatal aperture, rather than stomatal density, determine the long-term response of g_s to elevated [Ca].

Is transpirational water loss of C_3 and C_4 plants reduced in elevated atmospheric CO_2 ?

A recent study analysing historical records of continental river runoff found that runoff has increased through the 20th century. Using optimal fingerprinting statistical techniques Gedney *et al.* (2006) attributed this observation to a direct effect of increasing [Ca] acting to suppress plant transpiration following CO_2 induced partial stomatal closure. On a smaller scale, studies of tree-canopy water use of six deciduous tree species showed maximum reductions in tree transpiration under elevated [Ca] of 22% (marginally significant), but this only occurred at low VPD (Cech *et al.*, 2003). At the same site Leuzinger & Körner (2007) reported a 14% reduction in tree water use at elevated [Ca] over the growing season. Reductions in tree transpiration for sweetgum (*Liquidambar styraciflua*) exposed to elevated [Ca] ranged from 13–25% (Wullschleger & Norby, 2001; Schäfer *et al.*, 2002). Elevated [Ca] reduced canopy evapotranspiration by 22% for a C_4 dominated tallgrass prairie, but evapotranspiration in response to atmospheric CO_2 concentration was reduced by only 6–10% in elevated compared with ambient CO_2 conditions in a calcareous grassland (Owensby *et al.*, 1997; Stocker *et al.*, 1997). However, these studies are primarily chamber- as opposed to FACE studies. Ellsworth *et al.* (1995) measured sap flow in a *Pinus taeda* forest ecosystem exposed to FACE treatment and recorded only a marginal effect of CO_2 on canopy water loss, reducing transpiration by just 6–7%. They surmised that there was no evidence to suggest water savings in elevated [Ca] under drought and nondrought conditions Ellsworth, (1999) with the response being well within measurement error (Körner *et al.*, 2007). However, the observations of tree transpiration were only made for 8 days. Over such a short time scale it is

difficult to fully resolve the complex responses of leaf and canopy water use and their interactions with other environmental factors. Nevertheless, in irrigated conditions, Tricker *et al.* (2009) found whole-plant transpiration (measured by sap flux techniques for *P. × euramericana* grown as SRC in a bioenergy plantation at the popFACE facility in Italy) increased in elevated [Ca]. Similarly, a 2-year study of sap flux from trees growing in stands of pure aspen (*P. tremuloides*) and mixed aspen and paper birch (*Betula papyrifera* Marsh.) at the AspenFACE facility, USA, found sap flux increased in response to elevated [Ca] (18%) (Uddling *et al.*, 2008).

The response of canopy transpiration to elevated [Ca] is therefore very variable, ranging from reduced transpiration (Owensby *et al.*, 1997; Wullschleger & Norby, 2001; Schäfer *et al.*, 2002; Cech *et al.*, 2003; Leuzinger & Körner, 2007), through no significant response (Ellsworth, 1999; Cech *et al.*, 2003), to a considerable increase in canopy water use (Uddling *et al.*, 2008; Tricker *et al.*, 2009). Reference back to ‘The stomatal response of C_3 and C_4 plants’ would suggest that reductions in leaf-level g_s at elevated [Ca] are of greater magnitude than changes in canopy-level transpiration, making it clear that leaf-level measurements are not a reliable indicator of plant water use and that scaling results from the leaf- to the ecosystem-level can be a major source of error. The reduction in canopy transpiration of 13–25% for a sweetgum stand exposed to elevated [Ca] (Wullschleger & Norby, 2001) was associated with 31% reduction in g_s measured at the leaf-level for top of canopy sun leaves (Herrick *et al.*, 2004). Stomatal conductance measurements of leaves in a 12 year-old sweetgum plantation exposed to an enriched CO_2 atmosphere were up to 44% lower at elevated than ambient CO_2 , whereas canopy conductance averaged over the growing season was only 14% lower in the stands exposed to elevated [Ca] (Wullschleger & Norby, 2001; Wullschleger *et al.*, 2002a). Unlike leaf g_s , canopy conductance in this study was only marginally affected by CO_2 treatment. Similarly, Wullschleger & Norby (2001) determined a 28% increase in stand-level WUE for sweetgum trees grown in elevated [Ca], which was considerably less than the 50–75% increase in WUE calculated at the leaf-level (Wullschleger & Norby, 2001). Consequently, canopy-dependent responses to elevated [Ca] exist, producing a smaller effect at the whole-plant level than what may otherwise be predicted from leaf-level measurements of g_s and transpiration alone (Wullschleger & Norby, 2001) (Fig. 1). Thus, theories of ecosystem responses to drought in elevated [Ca], derived from early studies that inferred water use responses and how these translate into improved soil moisture conditions of entire ecosystems from leaf-level

g_s measurements, may over-estimate the benefits of growth in elevated [Ca]. It is evident that control of plant–water relations is complex, and at a larger scale canopy-dependent factors play a role in modifying the stomatal response to elevated [Ca] concentration. Examples of canopy-dependent factors are outlined below:

- (i) One documented response of growth in elevated [Ca] is an increase in leaf area index (LAI) for many species (Ferris *et al.*, 2001; Gielen & Ceulemans, 2001; Gielen *et al.*, 2001a, 2003; Taylor *et al.*, 2001b; Liberloo *et al.*, 2006; Uddling *et al.*, 2008). It is therefore possible for transpiration and water use on a larger scale to increase, offsetting the reductions in water use due to partial stomatal closure at the leaf-level. Observations of droughted cherry seedlings (*Prunus avium*) showed that while whole-plant WUE was increased by 56–103% with elevated [Ca], there was no difference in plant water use between CO₂ treatments, consequently there was no CO₂ induced enhancement of soil moisture content (Centritto *et al.*, 1999a). Tricker *et al.* (2009) found whole-plant transpiration for a poplar SRC plantation exposed to FACE treatment increased in elevated [Ca]; an average increase of 12% and 23% was observed in the first and second measurement campaigns respectively despite a 16–39% decline in g_s . In both these studies, leaf area was found to be higher in plants grown in elevated [Ca], which was thought to be partly responsible for the observations. Wullschleger *et al.* (2002a) conclude that equivalent rates of water use for plants exposed to ambient and elevated [Ca] suggest that there are trade-offs between increases in leaf area and reductions in g_s , such that in many cases there are few, if any, effects of elevated [Ca] on enhancing whole-plant water use.
- (ii) As stomata close and cooling via transpiration decreases, leaf and consequently canopy temperature have been found to increase (Wall *et al.*, 2001). This may have other microclimatic effects within the canopy such as decreasing humidity, thus increasing the leaf-to-air vapour pressure gradient. As a result, this may feedback to increase the driving force for transpiration, negating the CO₂ effect and leading to increased water use at the canopy scale (Wall *et al.*, 2001; Wullschleger & Norby, 2001). A number of studies have shown that stomatal responses to elevated [Ca] are only significant under high humidity (Wullschleger *et al.*, 2002a; Cech *et al.*, 2003; Leuzinger & Körner, 2007). Cech *et al.* (2003) for example found that the reduction in mean daily sap flux density of mature deciduous trees exposed to elevated [Ca] was almost negligible on days with high evaporative

demand. It remains unclear to what extent atmospheric feedback such as this will mitigate canopy-level CO₂ effects on plant water use (Leuzinger & Körner, 2007).

- (iii) The degree to which stomata exert control over transpiration is also governed by the aerodynamic conductance of the canopy. This is relevant for determining the response of plants to future global [Ca] concentrations as in situations where this exerts stronger control on water vapour loss than stomata, any potential change in g_s induced by elevated [Ca] will only marginally affect transpiration and stand water use (Wullschleger *et al.*, 2002b). Dense, uniform vegetation such as grassland has a low degree of atmospheric coupling whereas a natural forest stand, for example, which is more structurally diverse and open, allowing for greater air circulation within and above the canopy, is well coupled (Körner *et al.*, 2007b). In the latter case it is thought that the canopy will have a low aerodynamic resistance, with stomatal resistance being the dominant force controlling transpiration. In the former example with a high aerodynamic resistance, a number of feedback processes may compensate for reductions in g_s such that transpiration remains unaltered (Schäfer *et al.*, 2002).
- (iv) Canopy dynamics can alter the magnitude of response to elevated [Ca]. Stomatal conductance (g_s) has been found to vary with depth into the canopy. Leaves in the lower canopy are generally older, often possess lower nitrogen concentrations, and are therefore less physiologically active than upper-canopy leaves. Lower-canopy leaves also experience different environmental conditions in terms of radiation and humidity that can decrease maximal stomatal function. Wullschleger *et al.* (2002a), observed that g_s of mid-canopy sweetgum leaves was on average 30–40% lower than upper-canopy leaves, and no significant difference was observed for g_s between CO₂ treatment in lower-canopy leaves. Similarly, Gunderson *et al.* (2002) documented variation in g_s with depth into the canopy. Herrick *et al.* (2004) reported that g_s was reduced by a greater degree in sun leaves as opposed to shade leaves, which they suggest primarily reflects the lower light availability at points within the canopy.

Canopy-dependent effects that interact with elevated [Ca] to alter the magnitude of plant responses to an enriched CO₂ atmosphere clearly show the difficulty of predicting ecosystem responses to a changed climate in a naturally fluctuating environment. Bioenergy plantations will be particularly susceptible to canopy-dependent

factors because they are densely planted, relatively uniform systems. At full canopy closure both grass and tree plantations will likely display a low degree of atmospheric coupling allowing atmospheric feedback processes to maintain g_s and transpiration at levels unaffected by [Ca] concentration.

For bioenergy crop species, in relation to canopy dynamics, high levels of light interception throughout the canopy are identified as important to boost yield because it increases the photosynthetic capacity of the canopy (Dowell *et al.*, 2009). In this respect, the structure of the canopy is equally as important as leaf biochemical factors at determining the potential rates of photosynthesis that may be achieved at the leaf-level. Indeed, a study of photosynthetic characteristics of five polar genotypes found no correlation between net photosynthesis and biomass yield, concluding that rates of photosynthesis at the leaf-level may not be a yield-defining characteristic in *Populus* (Taylor *et al.*, 2001a). Studies of SRC *Salix* genotypes identified characteristics that increase the harvest and utilization of light as being better determinants of yield. The highest yielding genotype (Tora) was found to have higher LAI and a higher ratio of cell number: leaf area and higher leaf extension rates along with a considerably more open canopy than the lower yielding variety (Robinson *et al.*, 2004). Alternative growth strategies for high-yielding willow genotypes identified in another study were either (i) a large number of stems and low LAI and specific leaf area (SLA), or (ii) fewer, larger stems with higher LAI and SLA (Tharakan *et al.*, 2005). In any case, increased LAI due to elevated [Ca], apart from potentially contributing to increased overall canopy transpiration, as is observed in poplar SRC, may either increase yield as a result of greater photosynthetic capacity, through increasing the area for light absorption or increased substrate availability (i.e. CO_2), or reduce yield because of lower levels of light interception with depth into the canopy as a result of 'self-shading'. It is evident that canopies are complex structures with multiple microclimates and light-regimes depending on depth into, or position of, the canopy. Simple reductions in g_s with rising [Ca] concentrations can be dampened or accentuated depending on canopy structure, location and interaction with the environment, all factors contributing to the overall plant physiological response to elevated [Ca].

It is interesting to note that more recent FACE studies, that measure soil water content along with canopy transpiration, suggest that enhanced soil moisture content in elevated [Ca] is primarily a function of increased soil water holding capacity or reduced soil evaporation due to increased leaf-litter build up and fine root production, rather than a direct response to elevated

[Ca] driven through reduced g_s (Schäfer *et al.*, 2002; Uddling *et al.*, 2008). Additionally, these studies highlight different responses between species, with some saving water under elevated [Ca] and others not (Körner *et al.*, 2007). For example, in the study by Schäfer *et al.* (2002) sweetgum trees responded by reducing overall water use by 25% over the 3.5 year study whereas *P. taeda*, the dominant tree species, displayed no significant response. In the study by Cech *et al.* (2003), some species e.g. *Carpinus* were highly responsive to elevated [Ca], whereas others e.g. *Fagus* did not respond at all. Therefore, the magnitude of the CO_2 effect on stand transpiration will depend on the relative abundance of different species (Cech *et al.*, 2003), which will determine the degree to which a forest could be water-saving, if at all. While currently species abundance effects may be negligible for bioenergy crops grown as plantations with limited diversity, the importance of such interactions may increase in the future as high-diversity grasslands have been found to achieve higher bioenergy yields, up to 238% greater than monoculture yields (Tilman *et al.*, 2006).

Enhanced capability of soil water extraction in elevated atmospheric CO_2 ?

It is reasonable to suggest that in water stressed conditions plants may increase root biomass and architecture to extract water from the soil more efficiently and from greater depths. There is evidence for stimulated root production in elevated [Ca], especially fine roots which are important for water uptake, but whether this is a direct effect of [Ca] from increased carbon allocation to the roots, or that of overall enhanced growth rate in elevated [Ca] is less clear (Norby *et al.*, 1999, 2004; Körner *et al.*, 2005). Plant biomass (above and below ground) is found to increase under elevated [Ca] in many species (Ceulemans & Mousseau, 1994; Calfapietra *et al.*, 2003; Lukac *et al.*, 2003; Liberloo *et al.*, 2006). The majority of studies suggest there is no change in relative biomass allocation, i.e. the root:shoot ratio, indicating there is no greater proportion of carbon allocated to root production as opposed to above-ground biomass production (Norby *et al.*, 1995; Tissue *et al.*, 1997; Crookshanks *et al.*, 1998). Nevertheless, increased absolute root production in elevated [Ca], especially fine-roots, would improve water extraction by plants and allow access to greater water reserves that may be inaccessible to counterparts grown at ambient CO_2 concentrations.

Conclusion

When considering environmental impacts of bioenergy crops it is of concern that poplar SRC increases water

use in elevated [Ca], with similar responses likely for willow species under SRC management. If water availability is already reduced as a result of changing patterns of precipitation in elevated [Ca], increased water use of bioenergy crops has considerable implications for catchment water resources. In the case of poplar SRC (Tricker *et al.*, 2009) increased water used in elevated [Ca] was thought to be partly due to increased LAI. If, as studies have found with other species (Schäfer *et al.*, 2002; Uddling *et al.*, 2008), increased water use is also a result of higher soil water availability resulting from increased water-holding capacity of the soil or reduced soil evaporation, then the environmental implications may not be of such concern. However, a counter-argument may suggest that increased LAI along with delayed leaf senescence, also observed at elevated [Ca] (Taylor *et al.*, 2008), could increase the interception loss of canopies, reducing the amount of water actually reaching the ground during periods of rainfall and reducing the amount of time outside the growing season to replenish soil water reserves. This is an issue of much importance for bioenergy crop plantations, particularly the perennial species, requiring further investigation. Increased water use and LAI are indicative of increased yield, indeed higher biomass accumulation both above- and below-ground has been found in plants grown in elevated [Ca] concentrations (Ceulemans & Mousseau, 1994; Calfapietra *et al.*, 2003; Lukac *et al.*, 2003; Liberloo *et al.*, 2006), which will maintain high energy yields of these crops, helping to satisfy economic demands.

Plant responses to drought

Determining plant responses to soil water stress has always been important to our understanding of ecosystem function, and will be more so in a future climate with a predicted increase in the frequency of drought. Due to the shared stomatal pathway through which plant–water relations in response to both elevated [Ca] and drought are controlled, it is likely the two will interact, such that the response of one is mediated by the other. The effects of drought can be devastating on plant growth and survival. Photosynthetic CO₂ fixation is suppressed under drought stress by enhanced diffusive resistances within the leaf (closure of stomata and decline of mesophyll and chloroplast conductance) and by drought-induced impairments of metabolic processes (Tezara *et al.*, 1999; Lawlor, 2002; Flexas *et al.*, 2004, 2006; Yin *et al.*, 2006; Galle *et al.*, 2007). The combined effect of these leads to reduced carbon assimilation and ultimately a decline in plant growth (Chaves, 1991; Chaves *et al.*, 2002).

Cell enlargement is particularly sensitive to water deficit. Turgor pressure provides the driving force for cell expansion in growing cells and so with decreasing turgor potentials leaf area expansion is inhibited (Jones, 1992). Stomatal closure together with leaf growth inhibition prevents further water loss, which would result in irreversible cell dehydration and xylem cavitation during drought stress (Chaves *et al.*, 2003). As a consequence of reduced transpirational water loss and reduced leaf area however, the plant capacity to assimilate CO₂ is also reduced.

Productivity of perennial bioenergy crops species, both C₃ *Salicaceae* trees and C₄ grasses, is limited predominantly by water availability (Clifton-Brown & Lewandowski, 2000; Clifton-Brown *et al.*, 2002). Even within species, different genotypes display very different sensitivities to drought, which is important in crop breeding trials (Street *et al.*, 2006). A study of three genotypes of miscanthus, for example, indicated that while *M. sinensis* displayed stomatal regulation of water loss by reducing leaf conductance and photosynthesis in order to retain green leaf area, even under severe water shortage, *M. sacchariflorus* and *M. × giganteus* lost leaf area under drought by senescence (Clifton-Brown *et al.*, 2002). Differences in whole-plant WUE were not detected in the three genotypes (Clifton-Brown & Lewandowski, 2000) leading the authors to suggest that the 'best' strategy for drought survival would depend on the timing, frequency and magnitude of the drought; *M. sacchariflorus* and *M. × giganteus* would be better suited when droughts are normally short, however if droughts are prolonged, *M. sinensis* may be better suited being able to maintain leaf area and continue growth after the drought period has passed. Studies of C₃ *Salicaceae* trees indicate poplar is less responsive to water stress and changes in atmospheric vapour pressure than willow (Hinckley *et al.*, 1994; Johnson *et al.*, 2002), however variable responses to drought displayed by many poplar genotypes suggests a large pool of variation from which to select varieties with improved responses (Street *et al.*, 2006). Interestingly, research conducted by Ripley *et al.* (2007) suggests inherent differences between the C₃ and C₄ photosynthetic pathways with respect to the sensitivity of drought-induced metabolic limitations of photosynthetic activity. They suggest the C₄ pathway is more sensitive to metabolic inhibition, and this mechanism may partially explain the paradox of decreasing relative C₄ species abundance along regional gradients of declining rainfall, despite high WUE in C₄ leaves (Ripley *et al.*, 2007). This lends credence to the notion that a future temperate bioenergy landscape could be dominated by C₃ perennial bioenergy crop species such as poplar or willow.

The mechanism of stomatal closure in response to drought remains unclear. Stomata are observed to close in response to either a decline in leaf turgor and/or water potential, and low humidity (Chaves *et al.*, 2002). Experiments also show that stomatal closure is linked to soil moisture content. Therefore, it has been suggested that the stomatal control of transpiration in response to soil water deficit is mediated by a feed-forward signal from root to shoots, involving the chemical signal abscisic acid. For detailed reviews of plant responses to drought, readers are referred to Chaves *et al.* (2003) and Wilkinson & Davies (2002).

In conclusion, it is evident that fast-growing bioenergy crops are sensitive to drought, posing a serious problem for achieving yields that can attain and maintain required economic thresholds necessary for success of the bioenergy industry. Unlike first-generation biofuel crops that are mainly food crops such as maize, wheat, sugar cane and sugar beet (Gomez *et al.*, 2008), second-generation lignocellulosic bioenergy crops have not been supported by the necessary investment for years of work on breeding and improvement trials (Karp & Shield, 2008), having previously had minimal economic value. Nevertheless, recognition of poplar as a model tree (Taylor, 2002) and sequencing of the whole poplar genome represent significant advances (Tuskan *et al.*, 2006). Consequently, with continued investment in perennial bioenergy crop species there is the potential, given the large pools of genetic diversity, to develop improved genotypes with higher yield, lower water use and improved drought tolerance.

The interacting effects of drought and rising CO₂: does elevated atmospheric CO₂ confer drought tolerance during periods of water stress?

From studies that investigate directly the interaction between elevated [Ca] and water stress it is evident that the indirect effect of an enriched CO₂ atmosphere can improve survival of C₃ and C₄ species during periods of drought because of improved plant–water relations (i.e. reduced stomatal conductance, reduced canopy transpiration, improved plant–water potentials and increased soil water availability) (Morse *et al.*, 1993; Hibbs *et al.*, 1995; Baker *et al.*, 1997; Conley *et al.*, 2001; Ottman *et al.*, 2001; Wall *et al.*, 2001, 2006; Johnson *et al.*, 2002; Leakey *et al.*, 2004). Coupled with increased carbon gain, plant WUE is enhanced as a result of elevated [Ca], which is most pronounced under high soil water stress for many C₄ species. Early investigations by Nie *et al.* (1992) of the C₄ grass *Andropogon gerardii* found that transpiration under double ambient CO₂ concentration decreased significantly by 34% under high, and 41.9% under low soil water conditions. Studies of the C₄

grass *Amaranthus retroflexus* by Ward *et al.* (1999) showed that in response to increasing [Ca] concentration, *gs* and transpiration were significantly reduced. During a period of induced drought, plants grown at elevated [Ca] showed lower relative reductions in net photosynthesis by the end of the drought compared to plants grown at lower CO₂ concentrations, indicating [Ca] enrichment enhanced drought tolerance in this species (Ward *et al.*, 1999). Reich *et al.* (2001) investigating CO₂ × N (nitrogen) interactions in C₃ and C₄ grassland monocultures, found that overall, the percentage soil water was higher under elevated [Ca]. Over the growing season, *gs* responses to elevated [Ca], measured in maize (*Z. mays*) in a field FACE experiment where ample soil moisture conditions prevailed, showed that *gs* was on average 34% lower but photosynthesis was not stimulated (Leakey *et al.*, 2006). Nevertheless, this coincided with improved soil moisture availability (up to 31% higher) by midseason. In contrast, at the same site in 2002 when episodic droughts occurred, photosynthesis was stimulated, on average, by 10% (Leakey *et al.*, 2004), which was probably a result of increased soil water conservation in elevated [Ca] plots as found by the later study. In sugarcane plants, *Saccharum officinarum*, subjected to elevated [Ca] and an imposed drought, *gs* was reduced and net photosynthetic rate increased because of increased available soil water such that stressed plants in elevated CO₂ maintained *gs* and WUE at 20% and 74% of stressed plants grown at ambient CO₂ (Vu & Allen, 2009). FACE treatment of sorghum (*S. bicolor*) under ample and limiting soil water conditions was found to reduce plant evapotranspiration by 4% and 10% for dry and wet plots respectively, as averaged over the 2 experimental years (Conley *et al.*, 2001). Triggs *et al.* (2004) quantified evapotranspiration of sorghum exposed to FACE treatment and different soil moisture regimes over a 2-year study period. They found that while FACE reduced evapotranspiration from wet plots in both years, drought-stress resulted in reduced evapotranspiration from FACE plots in the first year (−8.5%) and increased evapotranspiration the following year (+10.5%). The authors suggested these plots had enhanced soil water availability for plants to continue transpiring during dry periods because the FACE-grown plants used water more slowly. This was supported by information on the sensible heat fluxes from the plots (Triggs *et al.*, 2004).

Recent literature for C₃ woody species is less abundant. Johnson *et al.* (2002) demonstrated that elevated [Ca] mitigated the effects of water stress in willow (*Salix sagitta*), but not in poplar (*P. trichocarpa* × *P. deltoides*). Whereas this could reveal a genuine difference in CO₂ × drought response between the species, it could

also be a factor of the genotypic dependency of the plant response, for example, poplar and willow species and their hybrids often show varying ability to regulate their transpirational water loss. Responses are therefore dependent on the genotype studied and can only be extrapolated to other genotypes/species with great caution. Nevertheless, in the absence of studies specifically on woody bioenergy crops, studies on other species can broadly indicate the likely outcomes.

Centritto *et al.* (1999a,b) found cherry seedlings (*P. avium*) subject to elevated [Ca] and drought increased total plant dry mass and displayed reduced transpiration rate per unit leaf area which contributed to enhanced WUE, but found no difference in actual water loss from the soil between CO₂ treatments, indicating no soil water conservation as a result of elevated [Ca]. Nevertheless, as is apparent from the results of Centritto *et al.* (1999a,b) and echoed by Wulschleger *et al.* (2002b) and Leuzinger & Körner (2007), studies that report *g_s* and evapotranspiration responses of plants grown in elevated [Ca] can only infer enhanced drought tolerance. Without data on the effect of CO₂ treatment on stand soil water status, or quantitative evaluation of the significance of any soil moisture effect on plant growth and physiology during drought, our understanding of how the indirect CO₂ effect impacts drought tolerance is little enhanced. Studies by Schäfer *et al.* (2002) and Uddling *et al.* (2008) that measured canopy transpiration along with soil water content shed more light on the indirect CO₂ effect, and interestingly find that although soil moisture content is increased in elevated [Ca], although not persistently, this is more an artefact of increased soil water holding capacity and reduced soil evaporation, rather than a direct response to higher [Ca] concentrations through reduced *g_s*.

Maintenance of higher (less negative) leaf water potentials are an important aspect to drought survival (Wulschleger *et al.*, 2002b). Centritto *et al.* (1999b) suggest that higher plant water potentials in drying soils enable plants to remain turgid and thus able to maintain metabolic processes, consequently increasing their tolerance to drought. Many studies document higher leaf–water and turgor potentials of plants in response to elevated [Ca] and under soil water stress. Tognetti *et al.* (2000) investigated field water relations of three Mediterranean shrub species under increasing seasonal drought at a natural CO₂ spring with elevated and ambient [Ca] concentrations. They found significant effects on plant leaf–water potentials. At sites with elevated [Ca], predawn and midday water potentials were higher (less negative) than those at control sites, with differences most pronounced between June and September when drought conditions were most severe. Deciduous forest trees in central Europe exposed to a

seasonal drought had less negative predawn leaf water potentials when exposed to elevated [Ca] (Leuzinger *et al.*, 2005). Johnson *et al.* (2002) found that predawn leaf–water potentials of poplar and willow species were higher in elevated than ambient [Ca], even as the imposed drought period progressed. At a given water potential, stressed trees in elevated [Ca] could maintain a more positive turgor potential than their counterparts in ambient CO₂. During the experiment wilting was observed (turgor potential = zero), but this only occurred in the control plots. Similarly, Hibbs *et al.* (1995), Morse *et al.* (1993) and Roden & Ball (1996), studying red alder seedlings, birch seedlings and eucalyptus species respectively, found that elevated [Ca] grown plants maintained high leaf–water potentials in the presence of soil water stress when compared to ambient CO₂ grown counterparts. Evidently, downregulation of photosynthetic capacity as a result of poor leaf–water status during drought is avoided in many species when grown in elevated [Ca].

Conclusion and perspectives

Although productivity of C₃ *Salicaceae* tree and C₄ grass bioenergy crop species is seriously compromised by soil water deficit, growth in an enriched [Ca] environment is shown in general to reduce leaf-level and canopy-level water use. These are often not of the same magnitude however, because canopy-dependent factors such as size, structure, dynamics and age-class become relevant at larger scales and alter the CO₂ response. As a result of these, poplar SRC water use is actually seen to increase in elevated [Ca] (Tricker *et al.*, 2009). Taken together, the literature on the effects of drought and elevated [Ca] on plant physiological responses suggests that under future climate changes of reduced precipitation and rising [Ca], bioenergy crop production will benefit from growth at higher [Ca] concentrations. Studies show that under drought, plants grown in elevated [Ca] maintain higher levels of net photosynthesis (Centritto *et al.*, 1999a,b; Ward *et al.*, 1999; Leakey *et al.*, 2004) and higher leaf–water potentials (Roden & Ball, 1996; Tognetti *et al.*, 2000; Johnson *et al.*, 2002; Leuzinger *et al.*, 2005) as a result of reduced *g_s* and transpiration (Nie *et al.*, 1992; Ward *et al.*, 1999; Conley *et al.*, 2001; Schäfer *et al.*, 2002; Cech *et al.*, 2003; Leakey *et al.*, 2004, 2006; Triggs *et al.*, 2004) and increased soil moisture availability (Schäfer *et al.*, 2002; Triggs *et al.*, 2004; Leakey *et al.*, 2006; Uddling *et al.*, 2008). However, some studies show increased canopy water use in elevated [Ca], including a bioenergy plantation of SRC poplar (Tricker *et al.*, 2009). Although soil moisture content was not measured in the bioenergy plantation, higher water use in elevated [Ca] has also been associated with higher soil water availability (Triggs *et al.*, 2004; Uddling *et al.*, 2008).

Recent research leads us to believe that higher soil water content under elevated [Ca], rather than being a primary response to elevated [Ca] concentrations through reduced plant water use, is more likely an indirect response which increases the water-holding capacity of the upper soil horizons and reduces soil evaporation, and is driven by increased organic matter input (King *et al.*, 2001, 2005; Schäfer *et al.*, 2002; Uddling *et al.*, 2008). Should this be the case for bioenergy plantations, it goes some way to assuage concerns about environmental impacts and economic productivity of bioenergy crops in future climate conditions. Particularly for the perennial bioenergy crop systems where, due to minimal disturbance of the rootstock for a period of many years (Karp & Shield, 2008), high organic matter is allowed to build up in the soil enhancing this water-holding capacity effect.

Studies have shown that, because trees are generally bigger in elevated [Ca], (i.e. bigger LAI, higher biomass (above- and below-ground), greater basal area (Ceulemans & Mousseau, 1994; Centritto *et al.*, 1999a; Calfapietra *et al.*, 2003; Lukac *et al.*, 2003; Liberloo *et al.*, 2006; Karp & Shield, 2008; Uddling *et al.*, 2008; Tricker *et al.*, 2009), they inevitably have a greater capacity for water uptake, which means the stomatal closure response to elevated [Ca] may be overridden and the effect of enhanced soil moisture availability will not necessarily persist throughout the growing season, especially if periods of drought are prolonged (Uddling *et al.*, 2008). Additionally, the response of increased soil water availability was observed in largely mixed stands of mature trees. Potential community structure effects on the over-all elevated [Ca] response of forests were discussed earlier ('Is transpirational water loss of C₃ and C₄ plants reduced in elevated atmospheric CO₂?') showing how some species in a mixed stand respond to CO₂ treatment and others do not. It is therefore the abundance of different species in a stand that will determine the water use of the entire forest, with species that do not respond or increase water use with CO₂ treatment being able to take advantage of water saving species (Schäfer *et al.*, 2002; Cech *et al.*, 2003; Körner *et al.*, 2007b). However, in perennial bioenergy plantations the situation is somewhat remote from a mixed stand, being a densely stocked stand of one species. In this situation, rates of water use may be exacerbated as all species are primarily high water users competing with each other to get a plentiful supply of available water. Indeed, rates of canopy water use were found to be higher in pure aspen stands than mixed aspen–birch communities (Uddling *et al.*, 2008).

Consequently, the response of perennial bioenergy plantations to the combined climate changes of elevated [Ca] and drought are complex and difficult to elucidate

from literature based largely on systems that bare minimal resemblance to managed, densely planted, rapidly growing, perennial bioenergy systems. Results of physiological responses to drought and elevated [Ca] can be obtained by looking at similar functional groups (i.e. C₃ v C₄) but as this review has shown, canopy structure, age and composition are also important and have a big influence on plant responses to elevated [Ca]. Hence, it is unclear how applicable the studies of mature forests are to rapidly growing, managed woody crops, and emphasises the need for studies that focus specifically on these crops.

Although often difficult and costly to initiate, this review highlights the need for multifactor, large scale FACE-type experiments specific for perennial bioenergy crop species that are the focus of this review. These experiments need to replicate the managed systems and community structure of bioenergy plantations and investigate growth responses to the many simultaneous effects of predicted climate change in these ecosystems. The interaction between drought and elevated [Ca] is certainly a climate change interaction that is not yet fully understood and requires further research. This would ideally include manipulation of droughts of different intensity and length, combining measurements of whole canopy water use using sap flux techniques with measurements of soil water content, and measuring growth characteristics including LAI along with canopy interception and through-fall, leaf-litter accumulation at the soil surface and organic matter content of the upper soil horizons. Basically a 'whole ecosystem' approach is needed to create a clearer picture of the many processes involved in determining the responses to climate change. Other changes in climate, that are forecast to accompany rising [Ca] levels, include elevated ozone (O₃) concentrations and higher temperatures. While the interaction between elevated O₃ and [Ca] has been studied in some species (Noormets *et al.*, 2001; Karnosky *et al.*, 2003), the interaction of temperature with elevated [Ca] has not been studied extensively (Long *et al.*, 2006), and very few experiments investigate anything more than a two-way interaction between elevated [Ca] and another climate change factor. This is understandable, given the limitations of time and resources; however the predicted effects of climate change will happen simultaneously rather than independently. The interaction between multiple climate variables and the consequences of this on plant growth and productivity therefore still remain much of a mystery today. Further, due to the variable responses to climatic stresses observed by different genotypes within a species and the importance of topography and soil conditions in modifying these responses, multisite and multigenotype experimental designs would also be an important component of further work,

especially to help with breeding and improvement trials to generate 'climate proofed' bioenergy crops.

Due to the difficulties and limitations that arise with multiple-factor field trials, maybe now the focus should be on integrating fieldwork and modelling to develop models that can effectively predict growth responses of bioenergy crop species to multiple climate changes. This would provide a more realistic understanding of plant and ecosystem responses to future climates resulting from different emissions scenarios, and the impact this may have on crop productivity. Modelling approaches that can predict the water use and yield of bioenergy crops in future climate scenarios would be invaluable to provide an evidence-based strategy for their large-scale deployment with minimal hydrological or environmental impact, and maximum economic gain.

It is clear that careful planning and location of bioenergy SRC and grass plantations remains paramount to maximize yield while minimizing negative hydrological and environmental impacts. Even if we imagine an ideal scenario where elevated [Ca] significantly improves productivity and drought tolerance through enhanced plant-water relations and soil water conservation, greater rooting depth and density produced as a result of elevated [Ca], and an extended growing season due to later senescence (Taylor *et al.*, 2008) means that these crops could still be potentially damaging at the ecosystem level in hydrologically sensitive areas. These issues have not been addressed in detail in this review; consequently it remains a further area requiring attention.

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