

## C3/C4 grasslands and climate change

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### Abstract

Species with the C3 and C4 modes of photosynthesis coexist in grasslands of North and South America, Central Asia, South Africa, Australia and New Zealand. In these ecosystems, the balance between C3 and C4 vegetation affects many functional aspects, including total and seasonal primary productivity and water use, the digestibility of herbage available to grazing animals, and the decomposability of litter and roots. Therefore, changes in the C3/C4 balance strongly influence the biogeochemistry and agronomic performance of these ecosystems, with potential impacts on animal productivity, carbon storage and nitrogen cycling. This review first presents the primary difference between the C3 and C4 photosynthetic pathways, and its consequences for plant carbon, water and nitrogen economies. Then, it explores how these physiological differences would influence the response of the C3/C4 balance to elevated CO<sub>2</sub> and global warming. Finally, expected responses are contrasted to the available evidence (from present-day and past-climate/vegetation studies, from analysis of recent regional changes, and from manipulative experiments) to verify their actual role in observed C3/C4 responses, and to identify gaps in our understanding of the mechanisms controlling the C3/C4 balance of grasslands.

Keywords: natural grasslands, elevated CO<sub>2</sub>, global warming, ecophysiological mechanisms, light use efficiency, maximum quantum yield, water use efficiency, nitrogen use efficiency

Abbreviations: CO<sub>2</sub> partial pressure ( $p\text{CO}_2$ ), Phospho-*enol*-pyruvate carboxylase (PEPCase), photosynthetic water use efficiency (PWUE), photosynthetic nitrogen use efficiency (PNUE), light-saturated photosynthetic rate ( $A_{\text{sat}}$ ), free-air CO<sub>2</sub> enrichment (FACE)

### Introduction

A unique difference between the C3 and C4 modes of photosynthesis determines contrasting photosynthetic responses to CO<sub>2</sub> and temperature in C3 vs. C4 plants, which in turn have consequences for plant's light, water and nitrogen use efficiencies, and for the digestibility and decomposability of plant biomass. As a result, the balance between C3 and C4 vegetation is believed to be potentially very responsive to climate change, and ecosystem function, potentially very responsive to changes in the C3/C4 balance.

Major C3/C4 ecosystems include savannas, in which a more or less dense population of C3 trees co-occur with a largely C4 herbaceous stratum, and grasslands, in which C3 grasses and forbs co-occur with C4 herbaceous plants, mostly grasses (Sage *et al.*, 1999). C3/C4 savannas occur in relatively hot, humid or subhumid climates (e.g. Brazilian *cerrado*, Argentinean *chaco*, tropical savannas in Africa and Northern Australia). C3/C4 grasslands occur either in temperate or subtropical climates with humid to subhumid conditions (e.g. *prairies* of North America, *pampas* and *campos* of South America, *grassvelds* of South Africa). A C4 component can also be present in grasslands of cooler and drier climates (e.g. *prairies* of North America, Mongolian *steppe*). Thus, even though the C4 flora comprises a relatively small number of species, mostly in the *Poaceae*, C3/C4 ecosystems include a substantial part

of global vegetation, and account for a relevant portion of its primary productivity (Lloyd and Farquhar, 1994).

Almost all C3/C4 grasslands and savannas operate as agroecosystems, supporting variable populations of grazing animals, typically sheep and/or cattle herds, managed rather extensively. Therefore, a better understanding of the mechanisms controlling C3/C4 interactions is important for the design and management of agroecosystems able to provide a seasonally stable supply of quality forage, and efficient at recycling nitrogen and storing carbon. Such agroecosystems exert a substantial influence on global biogeochemical cycles (Lloyd *et al.*, 1994), particularly in regard to the coupling of carbon and nitrogen cycles. Further, a better understanding of the controls of the C3/C4 balance in grasslands would improve our knowledge on the ecophysiological basis of species coexistence (Anten and Hirose, 2003), and hence of the controls of (functional) biodiversity. Moreover, because of the variable isotopic composition of CO<sub>2</sub> exchanged by mixed C3/C4 vegetation, a better understanding of the controls of the C3/C4 balance in grasslands will also contribute to a more accurate estimation of the isotopic signature of carbon fluxes required to constrain the partition of global carbon fluxes into terrestrial *vs.* oceanic components (Lloyd *et al.*, 1994; Randerson *et al.*, 2002).

This review focuses on C3/C4 grasslands, because this allows a more straightforward analysis of the influence of photosynthetic mode on the interaction between plants of similar morphology. Climate-change effects on C3 tree–C4 grass interactions have been reviewed elsewhere (e.g. Polley *et al.*, 1997; Scheiter and Higgins, 2009). Here, the intrinsic difference between the C3 and C4 photosynthetic modes is first presented, and its consequences for plants' carbon, water and nitrogen economies are described. Then, how such physiological differences could influence the balance between C3 and C4 species in response to increased levels of CO<sub>2</sub> and temperature is explored. Finally, expected responses are contrasted with available evidence (from present-day and past-climate/vegetation studies, from analysis of recent regional changes, and from controlled experiments) to verify their actual role in observed C3/C4 changes, and to identify gaps in our understanding of the mechanisms controlling the C3/C4 balance of grasslands.

### **Primary differences between C3 and C4 species: photosynthetic responses to temperature and CO<sub>2</sub> partial pressure**

The essential difference between the C3 and C4 modes of photosynthesis is that CO<sub>2</sub> partial pressure ( $p\text{CO}_2$ ) at the site of Rubisco is 5 to 10 times higher in C4 than in C3 photosynthesis. This effectively prevents photorespiration by suppressing O<sub>2</sub> competition, and also saturates Rubisco carboxylase activity. Since photorespiration is a temperature- and CO<sub>2</sub>-dependent process (Brooks and Farquhar, 1985), photosynthesis is higher in C4 than C3 plants at high temperature and low  $p\text{CO}_2$ .

C4 plants accomplish this *via* (i) a biochemical CO<sub>2</sub>-pump that involves Phospho-*enol*-pyruvate carboxylase (PEPCase), an enzyme with high affinity for CO<sub>2</sub> and no oxygenase function located in the mesophyll, and (ii) by concentrating Rubisco in bundle-sheath cells (Kranz anatomy; Hatch, 1987). Thus, PEPCase fixes CO<sub>2</sub> in mesophyll cells producing a four-carbon acid (hence the name). Fixed carbon is then translocated to bundle-sheath cells as malate or aspartate, where it is decarboxylated and the resulting CO<sub>2</sub> assimilated by Rubisco. Bundle-sheath cells have a low conductance for CO<sub>2</sub> diffusivity in C4 leaves. The reason is unclear, and may involve engrossed cell walls and/or lower membrane permeability to CO<sub>2</sub> (von Caemmerer and Furbank, 2003).

Pumping CO<sub>2</sub> is costly: each pumped molecule of CO<sub>2</sub> requires 2 ATP, produced by photophosphorylation at a rate of ~ 8 photons per ATP (Furbank *et al.*, 1990). The total cost of CO<sub>2</sub> pumping per unit fixed CO<sub>2</sub> is further increased by the fact that some of the pumped CO<sub>2</sub>

leaks back to mesophyll cells (Farquhar, 1983). Therefore, maximum quantum yield (moles of CO<sub>2</sub> fixed per mol of absorbed quanta at very low irradiance) is greater in C<sub>4</sub> than C<sub>3</sub> plants at high temperature or low intercellular *p*CO<sub>2</sub>, when photorespiration more than offsets ATP consumption by the C<sub>4</sub> pump. But the opposite becomes true at low temperature or high intercellular *p*CO<sub>2</sub> (Ehleringer and Pearcy, 1983).

The same interaction between photosynthetic mode and temperature is observed at high irradiances. This is because at high temperature light-saturated photosynthetic rate (*A*<sub>sat</sub>) is higher in C<sub>4</sub> than C<sub>3</sub> plants due to the photorespiratory load in C<sub>3</sub> photosynthesis, and to C<sub>3</sub> photosynthesis being progressively CO<sub>2</sub>-limited at increasing irradiance. Both these effects are avoided in C<sub>4</sub> plants because Rubisco operates at (near) saturating levels of CO<sub>2</sub>. At low temperature, Rubisco levels increase in C<sub>3</sub> plants and help maintain *A*<sub>sat</sub> high. In C<sub>4</sub> plants *A*<sub>sat</sub> decreases dramatically at low temperature, apparently because of a physical limit to the ability of C<sub>4</sub> leaves to increase Rubisco imposed by bundle-sheath cell space (Sage and Kubien, 2007), which leads to higher leakiness (Kubásek *et al.*, 2007).

### **Secondary differences between C<sub>3</sub> and C<sub>4</sub> species: nitrogen and water use efficiency**

The photosynthetic use efficiency of nitrogen (PNUE) is often higher in C<sub>4</sub> plants; that is, more CO<sub>2</sub> is assimilated per unit leaf nitrogen. This is because Rubisco, which accounts for a substantial part of leaf nitrogen, is fully engaged in CO<sub>2</sub> assimilation (as opposed to photorespiration) and operates (nearly) saturated by CO<sub>2</sub> (Bolton and Brown, 1980; Sage and Pearcy, 1987b). Moreover, the particular (Kranz) anatomy associated with the C<sub>4</sub> syndrome implies that C<sub>4</sub> leaves not only have less Rubisco (Ku, Schmitt and Edwards, 1979; Sage *et al.*, 1987) but also more lignified tissue (Dengler *et al.*, 1994). This is reflected in an inherently lower nitrogen concentration in C<sub>4</sub> than C<sub>3</sub> plants (Greenwood *et al.*, 1990), although Taylor *et al.* (2010) have argued this may be more related to phylogeny than to an inherent C<sub>3</sub> vs. C<sub>4</sub> difference.

The C<sub>4</sub> mode of photosynthesis also has indirect consequences for the use of water. The lack of oxygenase function and high affinity of PEPCase for CO<sub>2</sub> determines that C<sub>4</sub> photosynthesis saturates at intercellular *p*CO<sub>2</sub> as low as 150 μbar. As a result, low stomatal conductance, and thus low transpiration rates, do not affect C<sub>4</sub> photosynthetic rate (Ghannoum, 2009). Consequently, photosynthetic use efficiency of water (PWUE) is often higher in C<sub>4</sub> plants, that is, more carbon is fixed per unit transpired water (Ghannoum, 2009; Taylor *et al.*, 2010). Lower transpiration rates can lead to increases in leaf temperature (Ghannoum *et al.*, 2000), further improving the performance of C<sub>4</sub> relative to C<sub>3</sub> photosynthesis.

Comparative studies routinely report higher PNUE and PWUE in C<sub>4</sub> plants (e.g. Taylor *et al.*, 2010 and references therein). However, it is important to note that, first, such advantages are less, and eventually disappear, under severe stress (e.g. nitrogen: Sage and Pearcy, 1987a; water: Ghannoum, 2009), perhaps due to increased CO<sub>2</sub> leakage (Buchmann *et al.*, 1996; Meinzer and Zhu, 1998). Second, at low temperature PNUE and PWUE become lower than in C<sub>3</sub> plants because the lesser nitrogen content and stomatal conductance of C<sub>4</sub> plants do not compensate for their low photosynthesis rates (Schmitt and Edwards, 1981; Christie and Detling, 1982; Long, 1983; Sage *et al.*, 1987; von Caemmerer *et al.*, 2001). And third, saturating CO<sub>2</sub> increases both PNUE and PWUE in C<sub>3</sub> plants (Wand *et al.*, 1999), diminishing (even offsetting) the advantage of C<sub>4</sub> plants. Therefore, the actual relevance of secondary differences as determinants of the C<sub>3</sub>/C<sub>4</sub> balance depends, perhaps largely, on the primary difference between C<sub>3</sub> and C<sub>4</sub> plants in the response of carbon assimilation to temperature and CO<sub>2</sub>.

Quantum yield, PNUE and PWUE all measure the efficiency with which a resource is used during carbon assimilation. Of course, these are not the only mechanisms determining the

outcome of competition; the ability for resource acquisition is also important. Whether having the C3 or C4 mode of photosynthesis has specific consequences for plant morphogenesis and architecture that, in turn, affect the ability to capture light, nitrogen or water has been little studied, although improved PNUE has been suggested to allow C4 plants to develop a higher leaf area index in fertile sites and to partition more carbon towards roots in nitrogen-limited situations (Wedin and Tilman, 1996; Long, 1999). An isolated but revealing study by Werger *et al.* (2002) showed that excluding grazing from a C3/C4 grassland induced a change in species (tall grasses replaced short ones), but all stages of the succession were dominated by C4s –from the short *Zoysia japonica*, to the intermediate *Brachypodium sylvaticum* to the tall *Miscanthus sinensis*. Thus, at least in this subtropical site, any constraints on above-ground architecture causing species replacement were not C3- or C4-specific. Other outcomes may be the case when grass/forbs interactions are involved: because of the relatively minor number of C4 dicots, most forbs are in fact C3 species, and therefore factors influencing the forbs/grass composition of grasslands may also indirectly affect their C3/C4 balance.

### **Putative mechanisms controlling the response to climate change of the C3/C4 balance: comparison against the available evidence**

The two major trends associated with climate change, elevated CO<sub>2</sub> and global warming, have opposite effects on the quantum yield of C3 and C4 photosynthesis. Elevated CO<sub>2</sub> improves little photosynthesis in most C4 plants, but leads to higher quantum yield in C3 plants. Conversely, warming decreases the quantum yield of C3 plants. Ehleringer (1978) proposed that temperature plays a major role in determining the C3/C4 balance of grasslands because it affects light use efficiency in C3 species but not in C4 species. This hypothesis, hereafter the *quantum yield hypothesis*, is explicit in Ehleringer *et al.* (1997) and Collatz *et al.* (1998), who estimated the temperature at which the extra quanta required by C4 photosynthesis equals photorespiratory costs for a range of atmospheric *p*CO<sub>2</sub>. Thus, scenarios below such cross-over temperature are expected to be dominated by C3 species, and those above, by C4 species.

The photosynthetic responses behind the *quantum yield hypothesis* form the ecophysiological basis of the expected response of the C3/C4 balance to increased CO<sub>2</sub> and temperature (e.g. Long, 1999; Sage *et al.*, 1999; Wand *et al.*, 1999). Still *et al.* (2003) further extended it, arguing that the same interaction observed in quantum yield also occurs under light-saturated conditions: elevated CO<sub>2</sub> increases A<sub>sat</sub> much more in C3 plants than in C4 plants, and higher temperature increases A<sub>sat</sub> more on C4 than in C3 plants (A<sub>sat</sub> response to high temperature in C3 plants depends on the magnitude of CO<sub>2</sub>-diffusion limitation (Sage *et al.*, 2007)). Implicit in this view is that carbon gain is the main determinant of competitive outcome, which, of course, is a simplification of reality. But, as in the use of optimization theory in canopy models, it can be a null hypothesis to analyse the adaptive significance of photosynthesis-related characteristics of plants (Anten, 2005).

There has been considerable discussion about the actual value of cross-over temperatures. Although knowing the exact number may be of limited relevance for the interpretation of changes in the C3/C4 balance at regional or global scale, it is important to note that, actually, it is *p*CO<sub>2</sub> at the Rubisco site and leaf temperature, averaged over daytime hours of highest photosynthetic rates, that determine plant carbon assimilation. These quantities, often difficult to estimate, usually do not vary in the same magnitude than available proxies, typically atmospheric *p*CO<sub>2</sub> and mean air temperature. In what follows, the predictions of the *quantum yield hypothesis* are contrasted against available evidence from present-day and past-climate/vegetation relationships, from recorded modern (i.e. last 50 years) changes in C3/C4 balance of grassland ecosystems, and from results of elevated CO<sub>2</sub> manipulative experiments.

## **Present-day climate/vegetation relationships**

Variation in the C3/C4 balance of grasslands is often associated with some measure of temperature, which is typically taken as evidence of an overriding importance of the photosynthetic response to temperature (e.g. Long, 1999; Sage *et al.*, 1999). It also supports the idea of a seasonal niche separation for C3 and C4 species (Kemp and Williams, 1980), although strict separations seem rare (Turner and Knapp, 1996). Modelisation of the *quantum yield hypothesis* have shown a reasonably good agreement between predicted and observed latitudinal, altitudinal and seasonal patterns of present-day 'pure C3', 'mixed C3/C4' and 'pure C4' vegetation (Ehleringer *et al.*, 1997; Collatz *et al.*, 1998; Still *et al.*, 2003), lending support to the view that the interaction photosynthetic mode x temperature in carbon gain is a major determinant of the C3/C4 balance.

Exactly how good is 'reasonably good' has not been rigorously tested (Winslow *et al.*, 2003). In fact, the primary control of temperature and CO<sub>2</sub> is challenged by several authors, who usually claim a larger role for soil moisture in controlling the C3/C4 balance. Indeed, aridity is highly correlated with C4 abundance (Sage *et al.*, 1999 and references therein). However, it seems to act as a secondary control: C4 plants dominate grasslands in hot climates, either arid or humid, and the opposite is true in cold climates. However, in temperate climates, aridity does seem to help C4 vegetation to persist at temperatures that would otherwise have led to C3 dominance (Sage *et al.*, 1999; Cabido *et al.*, 2008). Why this is so is not completely clear. A cause put forward often is their higher PWUE. Another reason may be indirect effects on leaf temperature. Stomatal conductance is lower in arid than in humid environments, and irradiance and thermal amplitude, often higher, due to low cloudiness. This leads to higher daytime leaf temperatures (Ghannoum *et al.*, 2000), and could thus explain part of the effect of aridity on the C3/C4 balance. Cabido *et al.* (2008) suggested phylogenetic effects may also be involved.

A convincing case for the role of precipitation in controlling the C3/C4 balance was made by Winslow *et al.* (2003), who proposed that an inherent difference between C3 and C4 growing seasons is modulated by soil available water, and showed that, under such an assumption, the distribution of present-day C3/C4 biomass is closely related to the seasonality of rain. This is intuitively sound, since summer precipitation would favour the growth of C4 vegetation and thus increase its relative contribution to standing biomass and annual productivity. However, it can still be argued that it is temperature, not rain, which primarily determines the length and timing of C3 and C4 growing seasons. This contrast clearly shows the importance of distinguishing the time scale in discussing the factors controlling the C3/C4 balance of grasslands. Annual averages are always weighed by seasonal productivity and would thus be influenced by the seasonality of precipitation (or, for that matter, irradiance), while more instantaneous (i.e. monthly) averages would reflect more the temperature effect. An integrative model –conceptual or explicit– to interpret annual values would include a temperature function predicting the instantaneous C3/C4 balance, modulated by precipitation.

## **Past climate/vegetation relationships**

A primary role for temperature and CO<sub>2</sub> in determining the C3/C4 balance has also been argued in analyses of past climates. C4 vegetation underwent an abrupt expansion 6 to 8 million years ago, first in tropical climates and then in more temperate areas. Cerling *et al.* (1997) documented in detail this change in the diet of herbivores of Asia, Africa and North and South America, and associated such widespread process with atmospheric *p*CO<sub>2</sub> decreasing below 400 - 600  $\mu$ bar, arguing that a progressive CO<sub>2</sub>-starvation of C3 plants would also explain why the transition from C3 to C4 diet occurred first in hot climates (higher cross-over temperature).

This interpretation, consistent with the *quantum yield hypothesis*, is not, however, universally accepted. First, it is disputed that atmospheric  $p\text{CO}_2$  decreased during the expansion of C4 vegetation observed by Cerling *et al.* (1997) (Pagani *et al.*, 1999), since it seems it occurred much earlier (Pagani *et al.*, 2005). Second, it is claimed that such expansion was a transition from C3-forests to C4-savanna/grasslands ecosystems, driven by aspects regulating tree/grass interactions. Thus, in a phylogenetic analysis, Edwards and Smith (2010) propose that rain levels decreased below a threshold required to support rainforests, leading to the development of more open (well-lit, less shaded) canopies. In turn, Beerling and Osborne (2006) point out an increase in charcoal in sediments of more or less the same age as the C4 expansion, and suggest a feedback mechanism by which fires fuelled by accumulating C4 dead biomass enhanced the opening process. Thus, in a sense, Cerling *et al.* (1997) propose that grasslands expanded because they included C4 species, while Edwards *et al.* (2010), and to a lesser extent Beerling *et al.* (2006), suggest that C4 vegetation expanded because grasslands expanded.

While low-precipitation-plus-fire, rather than  $\text{CO}_2$  starvation, may indeed be the main cause of decreasing C3 forests, this does not explain why C4 rather than C3 grasses dominated the expanding grasslands and savannas. Temperature and  $p\text{CO}_2$  would still be the main determinants of the C3/C4 balance of these emerging ecosystems. Of course, both factors could have interacted: aridity would affect more and more quickly carbon-starved trees, because, for instance, their root mass ratio would have been lower, and their ability to reduce transpiration, limited by low  $p\text{CO}_2$ . An interesting question arise from these considerations: is low  $p\text{CO}_2$  more detrimental for the growth of C3-grasses than C3-trees?

A major role for temperature and  $\text{CO}_2$  in determining the C3/C4 balance is supported by analyses of more recent changes in Earth's climate. Atmospheric  $p\text{CO}_2$  has oscillated over the last half-a-million years, from 180 – 210  $\mu\text{bar}$  minima at glacial maxima to 280 – 300  $\mu\text{bar}$  during warmer interglacial periods. Modelling changes in C3/C4 vegetation following the last glacial maximum 15000 years ago for two sites that showed contrasting trends (increasing C3 forests in intertropical Africa vs. increasing C4 grasses in the steppes of central China), Flores *et al.* (2009) concluded that the C3/C4 balance was primarily sensitive to temperature and atmospheric  $p\text{CO}_2$ , and thus support the *quantum yield hypothesis*. Boom *et al.* (2002) went a step further and used the model behind the *quantum yield hypothesis* to actually infer past  $\text{CO}_2$  atmospheric pressure from C3/C4 data in Andean grasslands. This is a particularly interesting site because the lack of trees prevents confounding effects derived from tree/grass interactions.

### **C3/C4 changes modern grasslands**

Atmospheric  $p\text{CO}_2$  has risen steadily from *ca.* 280  $\mu\text{bar}$  in pre-industrial times (~1830) to *ca.* 390  $\mu\text{bar}$  today. This change is substantial enough as to make an impact on C3 vs. C4 growth, even if temperatures increased as much as 4 °C to 5 °C. In fact, temperature increases over the past century have been of lesser magnitude, and with a more restricted, regional scale. Similarly, precipitation changes differed in magnitude (and even direction) depending on the region. As a result, over the last hundred years or so, C3/C4 ecosystems experienced a uniform increase in  $\text{CO}_2$  availability but may have been exposed to distinct changes in temperature and precipitation. In spite of this apparently rich experimental material, few studies have analysed the recent evolution of the C3/C4 balance in modern grasslands.

This may be related to the fact that grasslands are poor at archiving, as compared for instance to forests. Comparing past C3/C4 composition of the Mongolian steppe inferred from soil organic carbon vs. present-day composition inferred from sheep hair (i.e. diet), Wittmer *et al.* (2010) found that specific areas of this region have become more rich in C4 vegetation. They ruled out potential effects of grazing and precipitation on this response, and concluded that it

was triggered by increases in summer temperature that overrode the advantage given to C3 plants by elevated CO<sub>2</sub>. Such a response, they argue, agrees with predictions from models based on the *quantum yield hypothesis*.

### Manipulative experiments

Atmospheric *p*CO<sub>2</sub> is predicted to increase continuously to reach ~ 600 μbar at the end of the present century. Experiments carried out in controlled environments indicate that such elevated level of CO<sub>2</sub> would favour plant growth more in C3 than C4 species, although the magnitude of the response is less than that predicted from leaf photosynthesis, and it is heavily modulated by nitrogen and water availability (Poorter, 1993; Wand *et al.*, 1999). Analysis of C3/C4 competition in mixed stands (well watered and fertilized) supports the view that the C3 component will increase under elevated CO<sub>2</sub> (Poorter and Navas, 2003). For instance, exposing turves of a New Zealand C3/C4 community in a growth room to a *p*CO<sub>2</sub> of 700 μbar for almost a year promoted a large increase of a C3 legume at the expense of the contribution of the dominant C3 grass and of *Paspalum dilatatum*, the only C4 grass (Newton *et al.*, 1995). Water stress, however, reduced the magnitude of this response (Clark *et al.*, 1999).

A few natural C3/C4 communities have been exposed to elevated CO<sub>2</sub>, for a number of years, using either field chambers or free-air CO<sub>2</sub> enrichment (FACE) set ups. Some of these studies would support the *quantum yield hypothesis*, but others would not. Thus, the aforementioned decline in *P. dilatatum* in New Zealand was apparent (albeit less marked) in a FACE experiment set up on essentially the same community (Newton *et al.*, unpublished). Further, in the North American Great Plains, elevated CO<sub>2</sub> accelerated the replacement of *Bothriochloa ischaemum*, a short C4 grass by several tall C3 forbs after exclusion of grazing in a humid site (range 200 to 560 μbar; Polley *et al.*, 2003). Likewise, in a semiarid shortgrass steppe, it increased the contribution of *Stipa comata*, a C3 grass, and of a C3 shrub (720 μbar; Morgan *et al.*, 2004a; 2007). But, on the contrary, elevated CO<sub>2</sub> decreased the presence of *Poa pratensis*, a C3 grass, in a C4-dominated humid tallgrass prairie (Owensby *et al.*, 1993), and enhanced more C4 than C3 growth in an Australian FACE experiment (Hovenden *et al.*, unpublished).

The reason for such a diversity of responses is not clear. In some cases, the expected increase in C3 photosynthesis was only transient (e.g. von Caemmerer *et al.*, 2001; Morgan *et al.*, 2001), a phenomenon usually referred to as 'acclimation'. Interestingly, most of these studies ascribed the observed responses to an elevated CO<sub>2</sub>-mediated enhancement of leaf water status rather than of photosynthesis itself. Indeed, a review of grasslands responses to elevated CO<sub>2</sub> concluded that *increasing atmospheric CO<sub>2</sub> induces water relations responses which in many situations dominate the system biomass and species responses to CO<sub>2</sub>, and could possibly be induced by moisture treatments alone* (Morgan *et al.*, 2004b).

That elevated CO<sub>2</sub> can improve soil water content has been demonstrated in several C3/C4 grasslands (Owensby *et al.*, 1997; Owensby *et al.*, 1999; Polley *et al.*, 2002; Lecain *et al.*, 2003). But why would improved water status benefit C3 species in some studies and C4 species in others? The results from this reduced set of studies suggest that, in humid environments, plant height can be important. In the tallgrass prairie, the C3 grass *P. pratensis* could not cope with increased canopy mass and height under elevated CO<sub>2</sub> in a grassland that was becoming less water-limited and in consequence more light-limited. In agreement, tall C3 forbs actually increased their contribution in this study (Owensby *et al.*, 1993). Paradoxically enough, in the short-C4 grass/tall-C3 forbs grassland, elevated CO<sub>2</sub> favoured C3 vegetation (Polley *et al.*, 2003). Naturally, this suggests that elevated CO<sub>2</sub> would strongly interact with grazing regime in these grasslands.

## Concluding remarks

The basic interaction in carbon gain between photosynthetic mode (C3 vs. C4) and  $p\text{CO}_2$  and temperature, as depicted in the *quantum yield hypothesis*, provides a baseline, a null hypothesis against which responses of the C3/C4 balance of grasslands can be contrasted. This hypothesis, which seems to adequately explain changes in the balance in C3 vs. C4 vegetation at geological time-scales, predicts that the C3/C4 balance should have moved during the last 50 years, and would continue to do so in the next 50 years, for grasslands in climates close to cross-over temperatures. More data recording the C3/C4 balance over the last 50 years of grassland ecosystems that experienced different (contrasting, if possible) changes in temperature and precipitation patterns should be useful to test these predictions.

The actual relevance of the mechanisms proposed by the *quantum yield hypothesis* is not universally accepted. Scepticism about the role of 'pure' photosynthetic responses in determining the C3/C4 balance is supported by recent results from elevated- $\text{CO}_2$  experiments carried out in natural grasslands. These suggest that improved water status, product of the reduced stomatal conductance observed in both C3 and C4 plants, and humid and semiarid ecosystems, plays a decisive role in determining the response of the C3/C4 balance to changes in  $p\text{CO}_2$  in the range 400 to 700  $\mu\text{bar}$ . This has two interesting consequences. First, if elevated  $\text{CO}_2$  does not generally promote C3 species, then it is likely that the C4 component of (some) C3/C4 grasslands will actually increase, driven by global warming. Second, it is not clear that the same relevance of water-mediated responses should not be apparent at lower  $p\text{CO}_2$ , say in the range 200 – 300  $\mu\text{bar}$ . If so, this would force a reinterpretation of the validity of the *quantum yield hypothesis* in explaining paleo-changes of the C3/C4 balance.

Finally, grazing is a factor that should be included in future studies (and interpretations) of climate change effects on the C3/C4 balance. It is a ubiquitous force that shapes grasslands through defoliation and nutrient cycling effects. For instance, in grasslands grazed at optimal or above-optimal stocking rates, and thus with a reduced scope for active selection by herbivores (Agnusdei and Mazzanti, 2001), any growth advantage given by  $p\text{CO}_2$ , temperature or water status to a particular functional group would be minimized by a correlative increased grazing pressure on it (Lemaire *et al.*, 2009). Up to now, only one experiment has explicitly analysed elevated  $\text{CO}_2$  effects on a grazed C3/C4 community (von Caemmerer *et al.*, 2001).

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