Will Elevated Carbon Dioxide Concentration Amplify the Benefits of Nitrogen Fixation in Legumes?[1]

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Growth at elevated [CO$_2$] stimulates photosynthesis and increases carbon (C) supply in all C$_3$ species. A sustained and maximal stimulation in productivity at elevated [CO$_2$] requires an enhanced nutrient supply to match the increase in C acquisition. The ability of legumes to exchange C for nitrogen (N) with their N$_2$-fixing symbionts has led to the hypothesis that legumes will have a competitive advantage over non-leguminous species when grown at elevated [CO$_2$]. On balance, evidence suggests that in managed systems, legumes are more responsive to elevated [CO$_2$] than other plants (e.g. Ainsworth and Long, 2005); however, in natural ecosystems, nutrient availability can limit the response of legumes to elevated [CO$_2$] (Hungate et al., 2004; van Groenigen et al., 2006). Here, we consider these observations, outline the mechanisms that underlie them, and examine recent work that advances our understanding of how legumes respond to growth at elevated [CO$_2$]. First we highlight the global importance of legumes and provide a brief overview of the symbiotic relationship.

IMPORTANCE OF LEGUMES

The majority of species capable of forming a symbiotic relationship with N$_2$-fixing bacteria belong to the Leguminosae family, and most of the roughly 20,000 legume species form a relationship with nodule-inducing bacteria, collectively known as rhizobia. The seeds of legumes have a high protein content, and some are also rich in oil. Consequently, legumes are an important source of protein and calories for humans and animals, exemplified by the fact that 25 countries plant 20% to 57% of their arable land with legumes such as Bambara groundnuts (*Vigna subterranea*), peas (*Pisum sativum*), beans (*Phaseolus vulgaris*), and lentils (*Lens culinaris*; http://faostat.fao.org/; Fig. 1). These include countries using intensive farming practices that produce mainly soybean (*Glycine max*), as well as developing countries where smallholder and subsistence farmers grow a wide variety of species that contribute to local food security. In addition, legumes are important components of animal forage in both temperate and tropical zones (Kretschmer and Pitman, 2001). In the tropics leguminous trees are used in rotation, or in multispecies cropping systems to enhance soil fertility, and as a source of wood. In all row crop, pasture, and agroforestry systems, the ability of legumes to fix N$_2$ reduces the need for chemical fertilizer inputs and provides important economic and environmental benefits (Peoples et al., 1995). Between one-third and one-half of the total N added to agricultural land is attributable to the legume-rhizobia symbiosis (Herridge et al., 2008; http://faostat.fao.org/). The contribution of legume-rhizobia symbioses to N$_2$-fixation natural ecosystems is hard to estimate, but is important to the composition and function of many terrestrial ecosystems, with woody legumes being particularly abundant in the tropics, while herbaceous legumes are widespread at higher latitudes (Vitousek et al., 2002).

THE SYMBIOSIS

The formation of a root nodule is preceded by an elaborate molecular courtship and the controlled infection of the plant host cell by rhizobia. Within the root nodule, a bacteroid, containing differentiated rhizobia, is isolated from the host cell by a peribacteroid membrane. In addition to forming and maintaining the root nodule, the host provides the bacteroid with a supply of C to fuel N$_2$ fixation. Suc is delivered to the root nodule via the phloem where it is cleaved by Suc synthase, enters glycolysis, and ultimately provides malate to the bacteroid. The bacteroid returns NH$_4^+$ to the host cell, which is assimilated into Gln. Further metabolism into other N-containing transport compounds uses C skeletons furnished by the host, and in the case of N-rich ureides, takes place in adjacent uninfected host cells. Both amides (Gln and
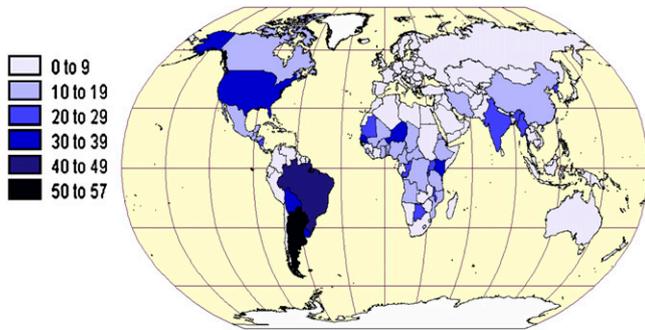
Figure 1. Percentage of agricultural land per country used for production of leguminous food crops in 2006. National crop production data were taken from the ProdSTAT database, Food and Agriculture Organization of the United Nations (http://faostat.fao.org), and included the following major crops: Bambara groundnuts, broad beans (*Vicia faba*), carob (*Ceratonia siliqua*), cowpeas (*Vigna unguiculata*), chickpeas (*Cicer arietinum*), dry beans (*Phaseolus* spp. and *Vigna* spp.), lentils, peanut, peas, pigeon peas (*Cajanus cajan*), and soybean.

Asn) and uriedes are exported from the root nodule via the xylem (Fig. 2). Within the bacteroid, malate is oxidized by the Krebs cycle to provide reductant for the nitrogenase complex and for the respiratory chain that fuels the nitrogenase complex with ATP. The outer layer of the root nodule forms a diffusive barrier that limits the entry of O_2 and protects the highly sensitive nitrogenase complex from O_2 inhibition. However, this barrier also limits the supply of O_2 to the bacteroid respiratory chain. The presence of high concentrations of leghemoglobin in the host cell, which gives active nodules their characteristic pink color, increases the flux of O_2 through the cytoplasm and controls the concentration of free O_2 available to the respiratory chain in the peribacteroid membrane (Crawford et al., 2000; Fig. 2).

ARE LEGUMES MORE RESPONSIVE TO ELEVATED [CO_2] THAN OTHER PLANTS?

The hypothesis that legumes have an advantage over nonleguminous plants in elevated [CO_2] is prevalent in the literature (e.g. Soussana and Hartwig, 1996; Zanetti et al., 1996; Serraj et al., 1998; Rogers et al., 2006). This hypothesis is well supported by numerous studies that report greater stimulation of photosynthesis and growth of legume species compared to other functional groups (e.g. Reich et al., 2001; Lee et al., 2003; Ainsworth and Long, 2005). Furthermore, experiments comparing nodulating and nonnodulating genotypes within a legume species have found that N fixers are more responsive to elevated [CO_2] than nonfixers (Nakamura et al., 1999; Luscher et al., 2000; Ainsworth et al., 2002, 2004). However, there is also significant variation in the magnitude of the response to elevated [CO_2] among legume species and genotypes (Reich et al., 2001; West et al., 2005), and

legume aboveground primary production was not statistically greater than that of other functional types when averaged across four Free Air CO_2 Enrichment (FACE) experiments (Nowak et al., 2004). A recent meta-analysis suggested that elevated [CO_2] will have no effect on N_2 fixation in many natural terrestrial ecosystems, which are characterized by intact soils, naturally occurring plant communities, and no fertilizer amendments (van Groenigen et al., 2006).

WHY IS ELEVATED [CO_2] PARTICULARLY BENEFICIAL FOR LEGUMES? THE UNDERLYING MECHANISMS

In C_3 plants, elevated [CO_2] increases photosynthesis and decreases stomatal conductance. All other effects at the plant and ecosystem level stem from these two primary responses and the secondary effects they elicit (Ainsworth and Rogers, 2007). In the absence of other nutrient limitations, legumes will be able to capitalize on the benefits of elevated [CO_2] by both increasing N_2 fixation and by reducing the negative impact of drought on N_2 fixation. In addition, legumes will be able to avoid or limit the few deleterious effects of elevated [CO_2] by reducing the potential for sink limitation of C acquisition and limiting the reductions in leaf and grain N content that are typically observed in C_3 plants grown at elevated [CO_2]. Here, we examine the mechanisms that allow legumes
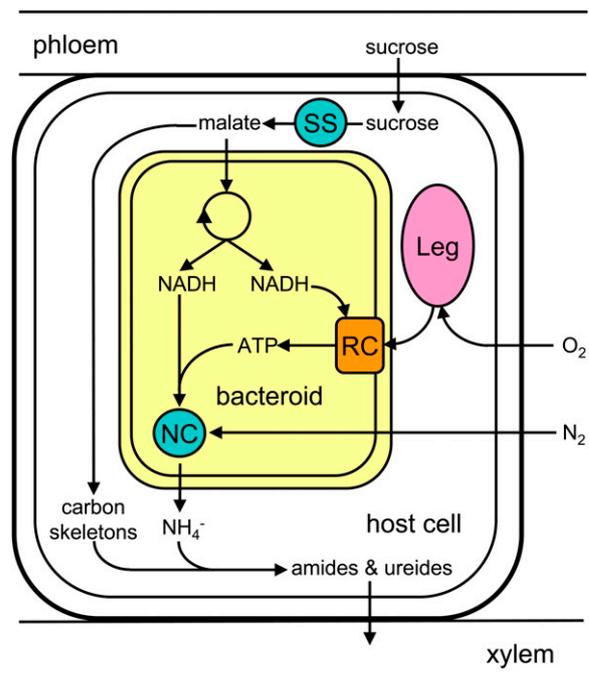
Figure 2. A root nodule showing the exchange of C and N between the host and bacteroid. Leg, Leghemoglobin; RC, bacterial respiratory chain; NC, the nitrogenase complex; SS, Suc synthase.

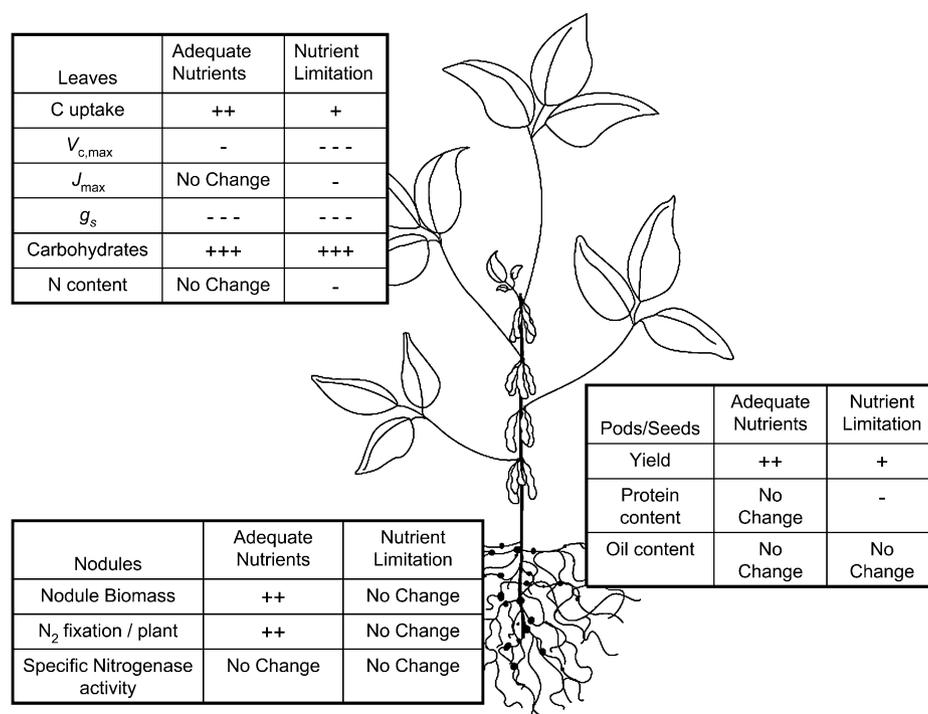to both capitalize on the benefits, and avoid, or limit, the disadvantages of growth at elevated [CO$_2$].

DO LEGUMES INCREASE N$_2$ FIXATION AT ELEVATED [CO$_2$]?

In fertilized crops and pasture species, the hypothesis that greater photoassimilate availability at elevated CO$_2$ supports stimulated N$_2$ fixation is broadly supported (e.g. Soussana and Hartwig, 1996; Zanetti et al., 1996; Ross et al., 2004; Rogers et al., 2006; Fig. 3). FACE experiments with soybean (*Glycine max*) and clover (*Trifolium repens*) showed a stimulation in photosynthetic C uptake and leaf carbohydrate content at elevated [CO$_2$] (Ainsworth et al., 2003b; Rogers et al., 2004; Leakey et al., 2009; Fig. 3). Increases in soybean aboveground biomass were not accompanied by reductions in tissue N content, implying that increased C supply was matched by additional N$_2$ fixation at elevated [CO$_2$] (Morgan et al., 2005; Rogers et al., 2006). An increase in the relative contribution of symbiotically fixed N to total plant N at elevated [CO$_2$] has been reported in a number of experiments (e.g. Schortemeyer et al., 2002; Lee et al., 2003), and in clover, the increased relative contribution of symbiotically fixed N$_2$ at elevated [CO$_2$] was a critical factor in maintaining the C to N ratio in the ecosystem as a whole (Zanetti et al., 1996). These experiments provide ample evidence that legumes increase N$_2$ fixation at elevated [CO$_2$].

An increase in N$_2$ fixation at elevated [CO$_2$] could result from an increase in nodule size, nodule number per plant, or an increase in specific nitrogenase activity, i.e. the amount of N$_2$ fixed per unit nodule and time (Fig. 3). Increased nodule biomass per plant is commonly reported in leguminous trees (e.g. Schortemeyer et al., 2002) and herbaceous species (e.g. Lee et al., 2003) grown at elevated [CO$_2$]. Changes in both nodule size and nodule number contribute to increased nodule biomass in different species and studies. On the other hand, there is less evidence that specific nitrogenase activity increases at elevated [CO$_2$] (Cen and Layzell, 2004), perhaps because many legumes lack excess specific nitrogenase capacity (Vance and Heichel, 1991).

In contrast to N$_2$ fixation in fertilized agricultural environments, N$_2$ fixation in natural ecosystems is not consistently stimulated by elevated [CO$_2$] (van Groenigen et al., 2006). Why? In some cases, stimulation of N$_2$ fixation by elevated [CO$_2$] may be species specific (West et al., 2005) or driven by the effects of elevated [CO$_2$] on competition and abundance of legumes in the ecosystem (Reich et al., 2006). In other cases, stimulation of N$_2$ fixation by elevated [CO$_2$] is limited by the availability of other essential elements (Reich et al., 2006; van Groenigen et al., 2006). For example, in a 6-year study of a N-poor calcareous grassland, phosphorus (P) limited the response of legumes to elevated [CO$_2$], which in turn limited N transfer to nonleguminous species in the ecosystem (Niklaus and Körner, 2004). A study of a Florida scrub-oak ecosystem reported that the initial stimulation of N$_2$ fixation in *Galactia elliottii* disappeared over time as molybdenum (Mo) became limiting in elevated [CO$_2$] (Hungate et al., 2004). Mo is a required cofactor for the

Leaves	Adequate Nutrients	Nutrient Limitation
C uptake	++	+
$V_{c,max}$	-	- - -
J_{max}	No Change	-
g_s	- - -	- - -
Carbohydrates	+++	+++
N content	No Change	-

Nodules	Adequate Nutrients	Nutrient Limitation
Nodule Biomass	++	No Change
N$_2$ fixation / plant	++	No Change
Specific Nitrogenase activity	No Change	No Change

Pods/Seeds	Adequate Nutrients	Nutrient Limitation
Yield	++	+
Protein content	No Change	-
Oil content	No Change	No Change

Figure 3. Summary of the effects of elevated [CO$_2$] and nutrient supply on legume leaf, pod/seed, and nodule characteristics. g_s, Stomatal conductance; $V_{c,max}$, maximum photosynthetic carboxylation capacity; J_{max}, maximum capacity for electron transport leading to ribulose-1,5-bisP regeneration. + indicates increases in elevated [CO$_2$]; − indicates decreases in elevated [CO$_2$]. The number of + or − symbols indicates the magnitude of change at elevated [CO$_2$].

nitrogenase complex, and elevated [CO_2] was thought to increase Mo adsorption to soil particles, decreasing the availability of Mo, and causing a systematic decline in N_2 fixation (Hungate et al., 2004).

These apparent nutrient limitations of N_2 fixation at elevated [CO_2] may not be specific to legumes or physiologically distinct from nutrient limitation in nonleguminous species. Mo limitation, which is largely confined to acidic soils, may impact N_2 fixation directly (Hungate et al., 2004). However, Mo limitation is also likely to affect plants dependent on soilborne nitrate because nitrate reductase also requires Mo cofactors (Crawford et al., 2000). Consider the P limitation of N_2 fixation; less than 1% of total plant P is found in nodules, and plants grown with a low P availability often have higher specific N_2 fixation (e.g. Almeida et al., 2000). Some crop species do need more P when they acquire N through N_2 fixation. However, in wild species, where the nutrient limitation of N_2 fixation has been shown to constrain the response to elevated [CO_2] (van Groenigen et al., 2006), the P requirement of legumes fixing N_2 is typically the same as when they acquired N from a mineral source (Vitousek et al., 2002). These observations support the view that the process of N_2 fixation is not limited directly by P availability and that whole-plant P requirement does not increase with N_2 fixation. Therefore, the observation that N_2 fixation, and the positive growth response of legumes, is constrained by a limiting P supply at elevated [CO_2] probably reflects a general P limitation that is exacerbated at elevated [CO_2]. The work conducted at the Swiss FACE experiment illustrates this well (Almeida et al., 2000). When clover was grown at a limiting P supply, nodule mass was decreased but specific N_2 fixation was higher. These plants also had a higher leaf N content, and a higher whole-plant N to P ratio than P-sufficient plants. When these plants were grown at elevated [CO_2], leaf starch content was increased. This indicates that plants grown at elevated [CO_2] and a limiting P supply were not limited by C or N supply. Rather, growth in low P limited the demand for N, resulting in fewer nodules and lower total N_2 fixation (Almeida et al., 2000). When P availability was increased, growth at elevated [CO_2] resulted in increased plant growth. The internal mechanism of response to limiting P under elevated [CO_2] is probably not unique to legumes. However, they may be unusual in the extent to which growth at elevated [CO_2] may help plants to release P from normally unavailable sources. The additional C and N_2 fixed at elevated [CO_2] by legumes can be partitioned to promote root growth, fuel mycorrhizal associations, increase the secretion of chelating agents, and increase the secretion of extracellular phosphatases (Vitousek et al., 2002; Houlton et al., 2008).

Therefore, the non-N nutrient limitation of the response of legumes to elevated [CO_2] is likely a general nutrient limitation of plant growth that is exacerbated at elevated [CO_2] where the availability of C, N, and potentially water is improved, rather than a specific nutrient requirement of legumes grown at elevated [CO_2].

ARE LEAF AND GRAIN QUALITY ALTERED BY GROWTH AT ELEVATED [CO_2] IN LEGUMES?

Reductions in leaf N content and or increases in C to N ratio are commonly observed in plants grown at elevated [CO_2] (Ainsworth and Long, 2005). Reduced leaf N content negatively impacts forage quality and can result in additional feeding by protein-limited insects as they attempt to compensate for the reduced leaf N content (Whittaker, 1999). In addition, a lower leaf N content, relative to the enhanced C supply at elevated [CO_2], limits the supply of N available for translocation to reproductive tissue during grain fill.

Evidence from mixed semigrassland communities managed as a forage crop showed that leaf N content and C to N ratio were unaffected by growth at elevated [CO_2] in legumes, but decreased in nonlegumes (Winkler and Herbst, 2004). In field-grown soybean, Rogers et al. (2006) showed that early season reductions in leaf N content at elevated [CO_2] disappeared once N_2 fixation was under way. These data provide good evidence that legumes that lack restrictions on their symbiotic exchange will not exhibit a reduced leaf N content at elevated [CO_2], and that the quality of leguminous forage crops will not be reduced (Fig. 3). There is evidence that this also helps legumes avoid compensatory feeding at elevated [CO_2] (Karowe, 2007; Lau et al., 2008). However, additional folivory at elevated [CO_2] was reported in soybean, and the response was linked to increased sugar content and a compromised defense system. This indicates that despite avoiding N dilution, increased damage by herbivores will likely occur in some legume species (Hamilton et al., 2005; Zavala et al., 2008, 2009). The response of herbivory to rising [CO_2] is highly complex and at this time a clear consensus of the response of herbivores to legumes grown at elevated [CO_2] is unclear.

Elevated [CO_2] will have direct effects on human health and well being through changes in grain quality. Protein content of nonleguminous C_3 grains including barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and rice (*Oryza sativa*) declined by 14% at elevated [CO_2] (Taub et al., 2008), and concentrations of calcium, sulfur, iron, and zinc were also significantly lower in wheat grain (Loladze, 2002). Will elevated [CO_2] affect legume seed quality to a similar extent? The evidence reported in two meta-analyses suggests that seed protein and N content will not be as affected by elevated [CO_2] in legumes as nonlegumes (Jablonski et al., 2002; Taub et al., 2008; Fig. 3). In soybean, growth at elevated [CO_2] significantly decreased protein content, but only by 1.4% (Taub et al., 2008). Averaging the effects of elevated [CO_2] on wild and domestic legumes, there was no effect on seed N

(Jablonski et al., 2002). Other important aspects of seed quality are also maintained at elevated [CO_2] in legumes. For example, percent oil in seeds of red kidney bean (*Phaseolus vulgaris*), peanut (*Arachis hypogaea*), mung bean (*Vigna mungo*), and soybean were unaffected by elevated [CO_2] (Burkey et al., 2007; Ziska et al., 2007; Thomas et al., 2009). The seed oil composition was altered by elevated [CO_2] in some species and experiments, but in general, results do not cause concern for rising [CO_2] effects on composition and edible quality of legumes (e.g. Burkey et al., 2007). Legumes can also provide key minerals, including iron, copper, manganese, calcium, magnesium, zinc, cobalt, P, and potassium, but there is very little data available to describe how mineral content of legume seeds changes at elevated [CO_2]. Theoretically, those minerals that are delivered to the plant through the transpiration stream, including P, may be less available at elevated [CO_2] but this phenomena would not be unique to legumes.

WILL LEGUMES MAXIMIZE PHOTOSYNTHETIC STIMULATION BY ELEVATED [CO_2]?

The sustained stimulation of C_3 photosynthesis by elevated [CO_2] increases the availability of carbohydrate and in doing so alters the balance of C and N resources. As a result the plant acclimates, adjusting its photosynthetic, respiratory, and N metabolism to improve performance under elevated [CO_2] (Leakey et al., 2009). The acclimation of photosynthesis is best understood in the context of the relationship between changes in intercellular [CO_2] (c_i) and the light-saturated rate of photosynthesis (*A*), otherwise known as the *A*-c_i curve (Fig. 4). Differences in the impact of elevated [CO_2] on photosynthesis among major plant functional types can explained on the basis of variation in the *A*-c_i relationship (Ainsworth and Rogers, 2007). The distinct effects of elevated [CO_2] on leguminous and nonleguminous C_3 herbs can be illustrated by comparing the responses of nonleguminous perennial ryegrass (*Lolium perenne*) and leguminous clover (Fig. 4). Perennial ryegrass grown at elevated [CO_2] displayed photosynthetic acclimation with a lower maximum photosynthetic carboxylation capacity ($V_{c,max}$) and a lower maximum capacity for electron transport leading to ribulose-1,5-bisP regeneration (J_{max}) when compared with plants grown at current [CO_2] (Ainsworth et al., 2003a). This results in a less-steep initial slope and lower asymptote on the *A*-c_i curve at elevated [CO_2] compared to current [CO_2] (Fig. 4A). As a result, leaves measured at an atmospheric [CO_2] of 600 μmol mol^{-1} (corresponding to a c_i of approximately 420 μmol mol^{-1}) had lower *A* if grown at elevated [CO_2] (Elev$_{600}$ in Fig. 4A) compared to plants grown at current [CO_2] (Curr$_{600}$ in Fig. 4A). As a consequence, the stimulation of *A* at an atmospheric [CO_2] of 600 μmol mol^{-1} relative to 360 μmol mol^{-1} (corresponding to a c_i of approximately 250 μmol mol^{-1}) was less in plants grown at elevated [CO_2]
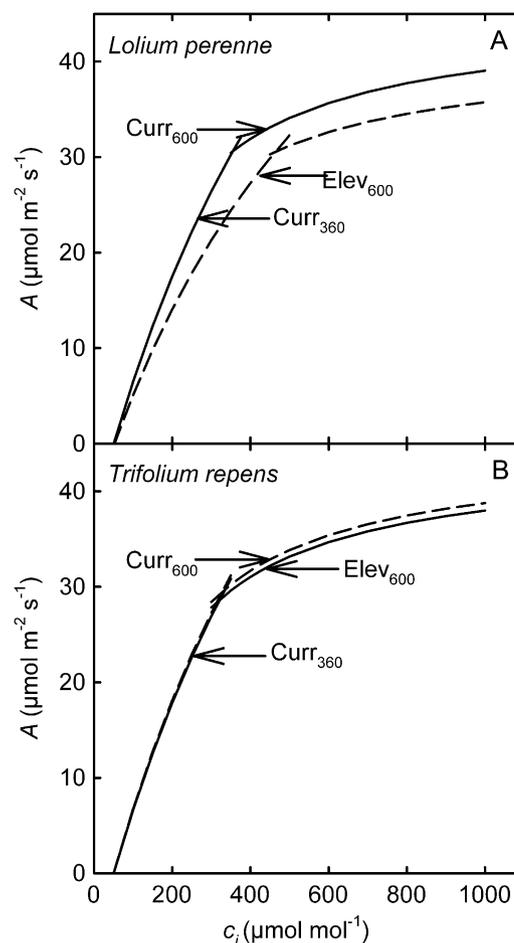
Figure 4. The response of photosynthesis (*A*) to internal [CO_2] (c_i) in perennial ryegrass (A) and clover (B) grown at current [CO_2] (approximately 360 μmol mol^{-1}, solid lines) and elevated [CO_2] (approximately 600 μmol mol^{-1}, broken lines) as part of the Swiss FACE experiment. Arrows indicate *A* at the c_i when leaves were: grown and measured at current [CO_2] (Curr$_{360}$), grown at current [CO_2] and measured at elevated [CO_2] (Curr$_{600}$), or grown and measured at elevated [CO_2] (Elev$_{600}$).

compared to plants grown at current [CO_2]. In contrast, clover did not photosynthetically acclimate to elevated [CO_2], maintaining the same $V_{c,max}$ and J_{max} at current and elevated [CO_2] and maximizing the stimulation of *A* by elevated [CO_2] (Fig. 4B; Ainsworth et al., 2003b). Legumes, such as clover, maximize the stimulation of productivity at elevated [CO_2] by avoiding photosynthetic acclimation because of their ability to reset the balance of C and N metabolism at elevated [CO_2]. Legumes avoid acclimation by allocating some of the additional photosynthate produced at elevated [CO_2] to their symbiosis with rhizobia to stimulate N_2 fixation. In nonleguminous species where N acquisition cannot be stimulated in this manner, C and N metabolism are balanced by reducing synthesis of Rubisco, which leads to lower $V_{c,max}$ and *A*, but also decreases the photosynthetic demand for the limiting nutrient, N, making it available to other sinks (Leakey et al., 2009). These

mechanisms maximize the efficiency of resource use for both functional groups, but explain how legumes can gain greater benefit from elevated $[CO_2]$ than nonlegumes.

The capacity for legumes to coordinate enhanced assimilation of C and N at elevated $[CO_2]$ to avoid down-regulation of photosynthetic capacity and ultimately maximize gains in productivity has been demonstrated across a range of species, environmental conditions, and ecological settings (Ainsworth and Rogers, 2007; Leakey et al., 2009). Nevertheless, there are situations where N_2 fixation cannot be stimulated in response to greater photosynthate availability at elevated $[CO_2]$ because some other factor is limiting. These include the nutrient limitations discussed above, as well as low temperature (Ainsworth et al., 2003b) and potentially drought stress. Unlike the response during warm spring weather (Fig. 4), low temperature was observed to cause photosynthetic acclimation to elevated $[CO_2]$ in clover (Ainsworth et al., 2003b). It seems likely that when some environmental factor prevents stimulation of N_2 fixation at elevated $[CO_2]$, excess photosynthate is likely to accumulate and lead to photosynthetic acclimation, but direct evidence of this mechanism in operation is needed.

WILL ELEVATED $[CO_2]$ HELP LEGUMES AVOID DROUGHT-INDUCED REDUCTIONS IN N_2 FIXATION?

Drought is a major limitation to crop production and causes a marked inhibition of N_2 fixation in legumes. This is particularly important in arid regions where legumes are also major food crops (Fig. 1). In some species, N_2 fixation is especially sensitive to even modest soil drying and reductions in N_2 fixation are observed before effects on transpiration or photosynthesis are apparent (Serraj et al., 1999). Despite considerable attention, the causes of drought-induced inhibition of N_2 fixation are still not well understood. There are a number of mechanisms proposed to account for the reduction in N_2 fixation under drought stress: (1) oxygen limitation, (2) C shortage, (3) regulation by N metabolism, and (4) oxidative damage of cellular components in the nodules (Serraj et al., 1999; Galvez et al., 2005; Ladrera et al., 2007; Marino et al., 2007; Naya et al., 2007). A detailed review of these mechanisms is beyond the scope of this *Update*. However, given that elevated $[CO_2]$ has previously been reported to ameliorate drought-induced reductions in N_2 fixation through a number of these mechanisms, we consider recent work in this area.

The supply of C to the bacteroid can limit N_2 fixation. Plants growing at elevated $[CO_2]$ should be able to enhance C supply to the nodule through increased photosynthesis. However, the drought-induced C shortage in the bacteriod occurs downstream of Suc supply. It is correlated with a crash in

host cell Suc synthase activity (Fig. 2), an accumulation of Suc, and a decline in malate content (Galvez et al., 2005). Therefore, it is unlikely that additional Suc available to host cell in plants grown at elevated $[CO_2]$ will be able to amielorate a drought-induced reduction in N_2 fixation. It is not clear if this mechanism is operating in all legume species and new evidence suggests that under moderate drought, impaired respiratory activity, and oxidative damage of nodule components precedes reductions in Suc synthase activity (Naya et al., 2007; Sassi et al., 2008).

Drought-associated decreases in N_2 fixation have been linked to increases in ureides, amides, and other amino acids in the leaf and nodule, and there is evidence for both a systemic and local feedback on N_2 fixation that is associated with N metabolites (Serraj et al., 1999). Growth at elevated $[CO_2]$ would provide more C skeletons to metabolize foliar ureides and amides, and the increased N sink at elevated $[CO_2]$ would stimulate ureide catabolism and reduce the leaf N-derived signal. However, recent work has shown that control is at the nodule level and does not appear to be mediated through a systemic N response (Ladrera et al., 2007; Marino et al., 2007). Since the availability of C skeletons required for the synthesis of N transport compounds is also impacted by the drought associated crash in Suc synthase activity, there is little potential for elevated $[CO_2]$ to increase C supply and alleviate drought-induced N metabolite accumulation in the nodules.

Elevated $[CO_2]$ perhaps has the most potential to help protect N_2 fixation from moderate drought by maintaining higher soil moisture content. There is overwhelming evidence that growth at elevated $[CO_2]$ reduces stomatal conductance (g_s; Fig. 3), and that this instantaneous response of stomates to CO_2 is maintained with time (Leakey et al., 2009). Recent evidence from FACE experiments, where the natural coupling between plants and the atmosphere is largely unaltered, also suggests that the decrease in g_s at elevated $[CO_2]$ often translates to a proportional reduction in canopy transpiration and an increased soil moisture content in a range of plants, including soybean (Leakey et al., 2009). Therefore, regardless of the mechanism by which drought impacts N_2 fixation, reduced g_s at elevated $[CO_2]$ may help prevent or delay reductions in N_2 fixation associated with dry periods by maintaining soil moisture content above inhibition thresholds. This will be particularly important for moderate drought stress where inhibition is readily reversed upon rewetting. Given that the reduction in g_s at elevated $[CO_2]$ is conserved across functional groups, legumes in mixed communities will also benefit from the increased water use efficiency of their competitors.

CONCLUSION

Current evidence suggests there are three key features of the response of legumes to elevated $[CO_2]$: (1)

unlike other nonleguminous C_3 plants, only legumes have the potential to maximize the benefit of elevated $[CO_2]$ by matching stimulated photosynthesis with increased N_2 fixation; (2) this potential can only be realized in the absence of limitations on productivity such as nutrient deficiency, low temperature, or drought; (3) rising $[CO_2]$ may offer some protection from drought-induced decreases in N_2 fixation, which will be become more prevalent with projected changes in precipitation intensity and frequency that are projected to accompany the rise in $[CO_2]$. However, despite the considerable importance of legumes to both agriculture and the function of natural ecosystems, there are still key knowledge gaps. There have been very few long-term studies of the response of field-grown legumes to elevated $[CO_2]$. This greatly limits characterization of the environmental conditions under which N_2 fixation can or cannot be stimulated at elevated $[CO_2]$. The feedback effects of nutrient limitation on N_2 fixation and photosynthesis have not been quantified. Only a single leguminous food crop (soybean) has been the subject of a fully open-air CO_2 enrichment experiment, and this study has not yet reported the effects of elevated $[CO_2]$ on N_2 fixation. No study we are aware of has quantitatively assessed the flow of C to nodules at current and elevated $[CO_2]$. These and other challenges create the prospect of many new and exciting findings in this subject area.

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