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Next generation of elevated [CO_2] experiments with crops: a critical investment for feeding the future world

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ABSTRACT

A rising global population and demand for protein-rich diets are increasing pressure to maximize agricultural productivity. Rising atmospheric [CO_2] is altering global temperature and precipitation patterns, which challenges agricultural productivity. While rising [CO_2] provides a unique opportunity to increase the productivity of C_3 crops, average yield stimulation observed to date is well below potential gains. Thus, there is room for improving productivity. However, only a fraction of available germplasm of crops has been tested for CO_2 responsiveness. Yield is a complex phenotypic trait determined by the interactions of a genotype with the environment. Selection of promising genotypes and characterization of response mechanisms will only be effective if crop improvement and systems biology approaches are closely linked to production environments, that is, on the farm within major growing regions. Free air CO_2 enrichment (FACE) experiments can provide the platform upon which to conduct genetic screening and elucidate the inheritance and mechanisms that underlie genotypic differences in productivity under elevated [CO_2]. We propose a new generation of large-scale, low-cost per unit area FACE experiments to identify the most CO_2-responsive genotypes and provide starting lines for future breeding programmes. This is

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necessary if we are to realize the potential for yield gains in the future.

Key-words: climate change; crop yield; FACE; genetic variation.

INTRODUCTION

The growing world population, increasing demands for grains for animal feeds, land loss to urban expansion and demand for bioenergy production are exerting more and more pressure on global agricultural productivity. Not surprisingly, the global food surplus is at a record low (USDA 2007). As global climate change increases average temperatures and alters the incidence of drought, global agricultural production will be profoundly impacted (Cohen 2003; Solomon *et al*. 2007). Therefore, a major challenge for plant biologists, agronomists and breeders will be to provide germplasm and seed material that maximize future crop production in a changing climate (Ainsworth, Rogers & Leakey 2008), while minimizing degradation of soil and water resources (Cassman *et al.* 2003) and limiting environmental impacts such as groundwater pollution and greenhouse gas emissions.

Atmospheric carbon dioxide concentration ($[CO_2]$) has risen from a pre-industrial concentration of ~280 to 384 μmol mol^{-1} in 2008 (Dr. Pieter Tans, NOAA/ESRL, www.esrl.noaa.gov/gmd/ccgg/trends), and could reach ~550 μmol mol^{-1} by 2050 and ~730 to 1020 μmol mol^{-1} by 2100 (Solomon *et al.* 2007). Even if the effects of various national and international programmes reduce emissions, the most optimistic stabilization concentrations for this century are between 450 and 550 μmol mol^{-1} (Solomon *et al.* 2007). This increase in $[CO_2]$ could provide a basis to offset losses in agricultural production caused by increased drought and temperature stress. However, it will be a major challenge to realize this increase because of the complex relationship between photosynthesis and crop growth and yield (e.g. Gifford & Evans 1981; Fichtner *et al.* 1993), alongside the complex interactions between plant growth and many other environmental factors. There is an increasing awareness that excessive use of nutrients and irrigation does not provide a sustainable strategy to increase crop yield. Further and major complications are introduced by future perturbation of global weather systems, which will result in changes in the temperature and water supply.

Higher $[CO_2]$ stimulates photosynthesis in C_3 crops because ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) is not CO_2 saturated at current $[CO_2]$ and because CO_2 inhibits photorespiration (Bowes 1991). In theory, at 25 °C, an increase in $[CO_2]$ from ~380 to 580 ppm could increase light-saturated C_3 photosynthesis of mature, sunlit leaves by 38% (Long *et al.* 2004). However, in practice, the average stimulation of photosynthesis in mature, sunlit leaves of wheat, rice and soy bean grown at elevated $[CO_2]$ (550–600 ppm) under field conditions [i.e. free air CO_2 enrichment (FACE)] falls short of the theoretical maximum (Long *et al.* 2004) and was only 14% on average across all FACE experiments (Long *et al.* 2006). This moderate stimulation of photosynthesis was in turn associated with limited gains in grain yield (13%; Ainsworth & Long 2005; Long *et al.* 2006).

In the limited FACE experiments on C_4 crops to date, there has been no significant stimulation of yield under well-watered conditions, because C_4 photosynthesis is saturated under ambient $[CO_2]$ (Wall *et al.* 2001; Leakey *et al.* 2004, 2006b; Long *et al.* 2006). However, all crops, both C_3 and C_4, potentially benefit from reduced demand for water. On average, stomatal conductance is reduced by 20% in plants grown at elevated $[CO_2]$ (550–600 ppm) in FACE experiments (Ainsworth & Long 2005). This reduces evapotranspiration, reduces soil moisture depletion and ameliorates stress during periods of drought (Kimball, Kobayashi & Bindi 2002; Leakey *et al.* 2004, 2006a,b; Morgan *et al.* 2004b; Nowak, Ellsworth & Smith 2004; Bernacchi *et al.* 2007).

Why should we focus on facilities for adaptation to elevated $[CO_2]$? Compared to temperature and water availability, $[CO_2]$ is unique in showing limited spatial variation. This means that it is not possible to exploit current adaptation to different climatic regions, and it is not possible to exploit existing differences in climate and soil to select for genotypes that respond best to elevated $[CO_2]$.

It could be argued that traditional breeding will have inadvertently increased CO_2 responsiveness over the past century as $[CO_2]$ has risen. If this were true, society might comfortably assume that over the next century, improved germplasm will acquire the desired responsiveness to $[CO_2]$ through routine selection for economic yield or general adaptation. However, in a study of four spring wheat cultivars, released in 1903, 1921, 1965 and 1996, the sensitivity of yield to $[CO_2]$ was inversely related to the year of cultivar release (Ziska, Morris & Goins 2004). Similarly, the average increase in yield for older spring wheat cultivars (released from 1890 to 1943) was greater than that of more modern cultivars (released from 1965 to 1988; Manderscheid & Weigel 1997). These studies and others (Amthor 1998) suggest that traditional breeding has *not* selected for $[CO_2]$ responsiveness, and indeed quite the opposite has occurred.

In view of the limited experimental evidence, further research is needed to elucidate the mechanisms of yield response to $[CO_2]$, to assess the genetic diversity available for improving responsiveness, and to devise efficient schemes for selection for adaptation to rising ambient $[CO_2]$, whether based on conventional plant breeding or systems biology approaches for selecting and engineering improved genetics. Testing the 'responsive' germplasm in different environmental conditions, such as under water stress or different temperatures or different soils, will be a crucial second phase of this research.

Climate change predictions indicate that drought and high-temperature stresses will increase throughout this century (Carter *et al.* 2007), directly damaging crops and making the timing of field applications of nutrients, herbicides or pesticides more difficult, thus reducing the efficiency of farm inputs (e.g. Porter & Semenov 2005; Tubiello, Soussana & Howden 2007). These deleterious aspects of climate change on crop systems may be offset in part by the beneficial effects of increased atmospheric [CO_2] on crop yield. Estimates of the potential benefit of elevated [CO_2] to global food supply suggest it will reduce the number of malnourished people in 2080 by between 12 and 580 million individuals, depending on the socio-economic scenario and on the crop models considered (Parry *et al.* 2004; Schmidhuber & Tubiello 2007).

The need to maximize the benefit of elevated [CO_2] and offset crop losses caused by greater water and temperature stress justifies a call for more experimental work investigating the [CO_2] responses of major food crops under representative field conditions. Crop response to [CO_2] is clearly a complex phenomenon, paralleling the complexity of crop responses to drought, salt stress or high temperatures. In order to dissect the mechanisms of response to complex traits, the use of molecular quantitative genetic tools is essential (Prioul *et al.* 1997; Tonsor, Alonso-Blanco & Koornneef 2005). We outline a plan for integrating physiology, genetics and modelling in a new generation of CO_2 experiments for crops. As described as follows, this requires experimentation at a scale not possible in the current FACE experiments. The plan is based on discussions from the workshop, 'FACEing the Future: Planning the Next Generation of Elevated CO_2 Experiments on Crops and Ecosystems', sponsored by the European Science Foundation, Interdisciplinary New Initiatives Fund (Rome, Italy; 5–7 December 2007). Because it may take 10–15 years to move from discovery of new advantaged genetics to commercial cultivars of annual grain crops, developing a robust strategy and supporting the planned work with the best possible facilities should be an urgent priority.

OBJECTIVES FOR THE NEXT DECADE OF RESEARCH

The present evidence indicates that conventional selection under rising [CO_2] has not succeeded in identifying genotypes that will perform well in even higher [CO_2] in the future; hence, identification of potential barriers and opportunities with respect to CO_2 responsiveness is critical. Barriers may not be limited to plant genetics, because feedbacks are not only at the individual plant level, but also at the system level, including the soil and atmosphere. Inevitably, the next generation of experiments will be limited in geographical scope. Based on total world grain production, rice, wheat, maize and soy bean are of most importance in terms of adaptation (Long *et al.* 2006), and are most intensively studied, but a number of other crops are of major importance, especially in developing countries. Therefore, a mechanistic framework will be necessary to generate improved models to project crop performance to a wider range of environments and species. Therefore, the next generation of elevated [CO_2] experiments with C_3 crops should: (1) quantify on a field scale the genetic variation for the grain yield response of major crops to elevated [CO_2], considering both inter- and intraspecific variation, and identify traits that may allow screening of a much wider range of germplasm; (2) use existing genetic variation and new tools from high throughput 'omics, comparative and quantitative genetics; molecular breeding; and bioinformatics to elucidate the mechanisms of crop yield response to [CO_2]; (3) in the longer term, determine how yield is impacted by elevated [CO_2] in combination with other aspects of climate change and shifts in agricultural practice, specifically rising temperature, altered water availability, rising tropospheric ozone concentration and altered nutrient availability. These are ambitious goals, but they can be met by a collaborative international effort among crop geneticists, molecular biologists, plant physiologists, agronomists and modellers. No less important are the engineers and technicians able to design appropriate experimental facilities, and assure their reliable and on-target operation.

APPROACH

The first step in meeting these objectives is to create facilities for field screening the yield response to elevated [CO_2] across a wide range of germplasm. Such facilities should be located in a major growing region for the crop(s) of interest. For example, a facility for rice might be located at the International Rice Research Institute (IRRI) in the Philippines, or in China, where nearly a third of the world's rice crop is produced (Coats 2003). A facility for soy bean might be located in the United States or in Brazil, and a facility for wheat in the major production areas of Australia, Europe, China, the United States, Canada or India. As economic and sustainable yield is the trait of interest, initial screening should occur under field conditions and management that reflect dominant agronomic practices and provide as natural an environment as possible. Furthermore, individual plots must be large enough to allow accurate yield estimates, and there must be adequate replication to ensure robust statistical interpretation.

These criteria argue for FACE facilities. A typical large-scale FACE apparatus consists of a number of 15- to 30-m-diameter plots within a field. Each plot is encircled by an array of pipes, which are suspended within and above the crop canopy (Fig. 1). CO_2 is released from pipes on the side of the plot which is upwind at any given moment. Wind direction, wind speed and the concentration of CO_2 are measured at the centre of the plot, with a computer-based feedback system that regulates the positions and amount of CO_2 released at different points around the plot (Hendrey *et al.* 1992, 1999; Lewin, Hendrey & Kolber 1992; Miglietta *et al.* 1997). Existing FACE systems operate continuously from crop emergence to harvesting, and maintain [CO_2] within the plot to within 10% of the target level for >90% of the time (Lipfert *et al.* 1992; Miglietta *et al.* 1997; Hendrey & Miglietta 2006). This is achieved with minimal perturbation of the soil–plant–atmosphere continuum.

The limitations of FACE technology have been extensively reviewed (Hendrey & Miglietta 2006). The major limiting factor for FACE is the cost of the large quantities of CO_2 that are released. The cost of this CO_2 varies dramatically between FACE experiments, depending on the final

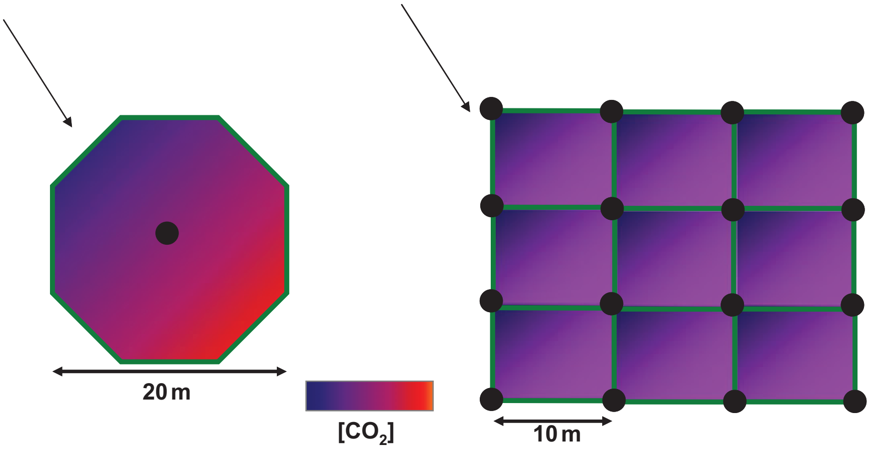

Figure 1. A typical distribution of [CO$_2$] across a free air CO$_2$ enrichment (FACE) octagonal plot or a hypothetical gridded FACE system. The arrows indicate the direction of the wind, and the color scale indicates the gradient in [CO$_2$] across a plot. The black circle indicates the location of a control box with a CO$_2$ analyser, anemometer and CO$_2$ regulator. The green lines represent pipes for release of CO$_2$.

concentration of CO$_2$, source of CO$_2$, plot volume fumigated, fetch, wind speed and uniformity of the vegetation. Therefore, there is no 'typical' FACE cost, and both the capital and operating expenses of a FACE experiment can vary by an order of magnitude depending on the location of the experiment and the factors mentioned.

The possibility to increase the scale and/or fumigation efficiency of FACE beyond the current levels is under investigation in alternative 'gridded' rather than linear designs (Fig. 1). A gridded system would also increase the flexibility of FACE by allowing additional modules to be added as needed without degrading the homogeneity of enrichment. A potential downside would be slightly impaired access to the crop plants.

Another solution to reducing FACE operating costs is to identify lower-cost sources of CO$_2$. Geological CO$_2$ sources from natural wells occur around the world; large CO$_2$ wells exist in the United States (e.g. in Arizona, Colorado, Mississippi, New Mexico, Utah and Wyoming) and in Europe (e.g. at Répcelak and Oelboe in Hungary; at Bad Driburg-Herste and Rottenburg in Germany; and in France, Spain and Italy). Some CO$_2$ wells are capable of producing more than 800 tons of CO$_2$ per hour (Heinicke *et al.* 2006), and new strategies for detecting additional geothermal systems have been investigated in detail (Lewicki & Oldenburg 2004). However, CO$_2$ is not the only gas emitted from natural vents. Concentrations of methane and hydrogen sulphide are often much higher than ambient atmospheric concentrations (Heinicke *et al.* 2006). If technology exists to scrub dangerous contaminants at a reasonable cost, this may be a viable source of CO$_2$ for experimentation. Unfortunately, few geological sources have been identified within the major growing areas of the major grain crops. Recent technological advances have been made in CO$_2$ sorbents than can capture CO$_2$ directly from the atmosphere (Zeman & Lackner 2004; Zeman 2007). If the CO$_2$ can be released from them at low cost, this might provide another viable source for FACE in the future. Alternatively, fossil fuel power stations and alcoholic fermentation for producing biofuels release large quantities of CO$_2$ (Kheshgi & Prince 2005; Yang *et al.* 2008). Fermentation, unlike power plants, is particularly attractive because the gaseous by-product is near pure CO$_2$. Placing FACE facilities next to fermentation facilities is an attractive opportunity, because many of these are located within grain-producing regions. It is equally important that such a facility be close to a large academic or research institution with expertise in plant sciences and specifically grain crop improvement. FACE facilities will not only need trained personnel for plant growth and facility maintenance, but also to manage site access, organize and coordinate the needs of large teams of scientists, and to provide an infrastructure for data acquisition, storage and analysis. This will represent a large component of the fixed costs in a large FACE site.

FACE experiments have traditionally been used to investigate the response of crops grown at current and elevated [CO$_2$]. The effect of elevated [CO$_2$] on physiological and biochemical parameters of interest is typically <25% (Long *et al.* 2004), and changes in gene transcript abundance are rarely greater than ~twofold (e.g. Ainsworth *et al.* 2006). Differentiating between the yield responses in germplasm and identifying the physiological and molecular responses that underlie those differences will require increased statistical power. New FACE facilities can increase statistical power by increasing the number of plots and utilizing innovative experimental designs. However, maximizing the uniformity of growth conditions will be a key challenge for reducing variation, so careful site selection for uniform nutrient and water availability and topography will be critical. The current design of FACE sites adequately controls the variation in [CO$_2$] (Hendrey *et al.* 1997), but improved performance and reliability will aid detection of small but physiologically important effects. With the existing ring design, control of [CO$_2$] degrades with increase in plot size. Any design for a new, large-scale fumigation method will need to be achieved without reducing the spatial and temporal uniformity of fumigation. This is the reasoning behind the modular gridded design proposed here, which in theory will allow an increase in scale without reducing control (Fig. 1).

OPTIMIZING THE PREDICTIVE POWER OF FACE

FACE systems are often considered expensive, but the net cost is compensated for by economies of scale, and the cost per unit ground area is considerably less than alternative systems (Hendrey & Kimball 1994). Nevertheless, it is critical to maximize the power of the experimental design. In the past, the primary experimental aims have been to characterize the impacts of climate change on yield and investigate response mechanisms of single genotypes. However, we note an urgent need to move beyond assessing climate change impacts and to develop strategies for adaptation, that is, identifying how crops can be selected to increase their yield response to rising [CO_2]. The initial FACE experiments required a large area of uniform vegetation; the new research requires investigating large numbers of genotypes. Current FACE experiments partially address these conflicting needs by allocating half of a treatment plot to genotype trials, and the other half to investigate processes of a single genotype (Ort *et al.* 2006). This current approach only allows sufficient space to examine the yield of up to ~20 genotypes in a 20-m-diameter FACE plot. To place this in perspective, to investigate the association of CO_2 responsiveness with a single quantitative trait locus (QTL) mapping population, approximately 150 inbred lines would need to be investigated. For example, a recent QTL analysis of drought tolerance in rice used 154 lines (Lanceras *et al.* 2004). If each of 150 lines was planted in a 2×2 m space, the experiment would require a treatment plot of more than 600 m^2, which includes a 1 m border adjacent to the release points that would not be used for sampling. Association mapping will require similar or even larger populations, especially if panels of cultivars are complemented by using segregating populations to break population structure. Current treatment plots in crop environments are ~20 m in diameter, and a larger diameter plot would suffer from marked [CO_2] gradients, which in itself would be solved only with more replications. It would appear that a gridded system (Fig. 1) could exceed this scale without these problems, but gridded systems remain to be tested.

In future crop FACE systems, physiological and molecular phenotyping technologies should be used to analyse large populations of genetically diverse and genotypically characterized plants. This is a crucial advance compared to the past, where at best, only a small number of genotypes were compared. Past experiments provided descriptive information, but did not allow rigorous genetic dissection and analysis of inherited variation in response to elevated [CO_2]. Functional genomics and quantitative genetics with populations of plants will allow us to causally dissect the complex, multifactorial network that controls carbon allocation, growth and yield. This information could open up new perspectives to understand the genetic and molecular basis of the response of plant growth to elevated [CO_2]. The proposed approach will generate a homogenous data set that documents the response of yield, and many physiological and molecular parameters across a large population of genotypes in elevated [CO_2]. This data set will be a powerful resource to develop mechanistic plant growth models, and to perform multivariate data analysis to identify parameters that influence the relationship between elevated [CO_2] and growth. The approach outlined here will pinpoint hypotheses about the underlying mechanisms, which can be tested by detailed analyses of small sets of plants, including near isogenic lines (NILs), that is, lines with different alleles at one or a few loci in a common genetic background. This approach will support QTL mapping, either via association mapping or in combination with the use of inbred populations.

On a pragmatic level, there are important questions relating to selection of germplasm and, in a broader sense, the exploitation of biodiversity to maximize crop yield in a future high [CO_2] world. Plant breeding uses phenotypic characters and genetic information to identify useful germplasm, which is crossed to create populations that are then grown and scored for important traits. Breeders are unable, however, to identify or select material that responds well to elevated [CO_2], because they have to grow their material at current [CO_2]. One important aim will be to learn whether any traits for which breeders are currently selecting affect, either positively or negatively, the response to elevated [CO_2]. We also need strategies to prioritize lines for screening in elevated [CO_2].

A novel approach is to build on the multilayered data sets that will be generated in FACE facilities. The results from a test population (50–100 genetically diverse genotypes) could be analysed by multivariate statistical methods to identify parameters whose values in ambient [CO_2] correlate with the yield response in elevated [CO_2]. These parameters could then be used to survey large genetic populations and predict which genotypes should show a particularly strong or weak response to elevated [CO_2]. In an iterative cycle, they would be grown under elevated [CO_2] in the FACE system to test the quality of the predictions and refine the parameter set that is used for the prediction. While it may be possible to pre-select genotypes based on pre-existing information about their responses to water, nutrient supply or temperature, it will also be important to generate parallel data sets at ambient [CO_2] in the FACE facility. In such a comparison, it will be necessary to concentrate on parameters that can be measured cheaply and easily, for example, plant architecture and phenology, stable isotopes and nutrient and metabolite levels. Integrative parameters should be included that are measured by plant breeders, like yield in different agronomic regimes at ambient [CO_2] (e.g. under altered fertilization, water supply or temperature). This would increase the speed with which large populations can be presorted and cycled through FACE facilities to assess their response to future [CO_2]. In addition to developing predictors for a given crop, this approach will also reveal similarities and differences among species. An important implication of this strategy is that future FACE sites would need to have a much larger area under ambient [CO_2] than under elevated [CO_2], at least for the first years of operation. Where appropriate, parts of the facilities might be located at multiple sites to exploit natural climatic or edaphic gradients.

UNDERSTANDING INTERACTING ELEMENTS OF GLOBAL CHANGE

Further research is needed to extend understanding of crop responses to climate change across a broad range of environmental conditions. A new Australian FACE experiment with wheat incorporates ecophysiological modelling, and is taking the approach of varying planting date, water supply and location in order to study how elevated [CO_2] will interact with both higher temperatures and lower water availability. Future FACE experiments should also manipulate environmental factors other than [CO_2] to ensure that selection for improved responsiveness to [CO_2] is not at the cost of tolerance to other features of global climatic and atmospheric change, notably increased temperature, ozone and drought incidence.

Here, two levels of interactions should be distinguished; firstly, if there is any correlation between the response to elevated [CO_2] and the response to another variable and, secondly, if there is an interaction between elevated [CO_2] and the other variable. The first can be approached by combining information about the response in single-factorial experiments, as outlined in the last section. The second will require multifactorial experiments, with simultaneous variation of elevated [CO_2] and the other variables. For practical and financial reasons, the latter can only be done in a second stage, using a smaller number of prioritized genotypes.

FACE facilities allowing multifactorial experiments would be critical for testing germplasm produced by combining tolerance of these changes in other environmental factors with responsiveness to [CO_2]. In addition, these facilities would provide data on the interactions of temperature, drought, ozone and [CO_2] to better inform yield prediction models. Interactions between elevated [CO_2] and crop stress factors such as heat, drought or ozone could be investigated using complementary methods such as infrared heater arrays for warming ecosystem field plots (Kimball *et al.* 2008), passive infrared night-time warming and rain exclusion systems (Mikkelsen *et al.* 2008) and open-air ozone enrichment (Morgan *et al.* 2004a; Karnosky *et al.* 2007).

RESEARCH PRODUCTS

What are the expected outcomes from this new generation of research? We anticipate that within a decade, the proposed research would identify: (1) germplasm with high yield responsiveness to elevated [CO_2] in a changing climate; (2) the most appropriate parental materials for crop improvement programmes; and (3) potential feedbacks between new cropping systems and the environment. Improved mechanistic understanding of plant response to elevated [CO_2] will be achieved by combining quantitative genetics with molecular and biochemical phenotyping, and general agronomic and biogeochemical understanding of responsive germplasm. This approach will also enable development of new screening tools and application of biotechnological approaches to improving yield in addition to conventional breeding. While significant progress has been made in recent years in using climate model predictions with ecophysiological models applying different methodologies (Hansen & Jones 2000; Hansen *et al.* 2006), the underlying processes involved in allocation of assimilate to various plant components and their responses to changing [CO_2] are still not well understood. Thus, the new generation of FACE research also must better inform models so that they can be used with confidence to explore the impacts of different global change scenarios or to guide decision making by producers, policy-makers and other stakeholders.

From the ecological perspective, crop systems are simple systems that provide important platforms for testing broader hypotheses on ecosystem responses to atmospheric change. Linking crop system responses to ecosystem modelling can then be used to develop strategies to inform land managers about appropriate adaptive strategies and policy-makers about future resource management issues. Therefore, a new generation of FACE experiments with crops will contribute to a more holistic understanding of ecosystem responses to elevated [CO_2].

CONCLUSIONS

The next generation of FACE experiments should investigate the world's major grain crops in representative production areas, where a highly qualified group of staff and scientists can maintain the facility. Given the cost of FACE, it will be important to take advantage of sources of low-cost or free CO_2. The scale of the FACE experiments must be sufficient to deal with a minimum of 150 genotypes per growing season. This generation of experiments would focus on *adapting* crops to the future environment, specifically elevated [CO_2], using the tools of molecular genetics.

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REFERENCES

Ainsworth E.A. & Long S.P. (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* **165,** 351–372.

Ainsworth E.A., Rogers A., Vodkin L.O., Walter A. & Schurr U. (2006) The effects of elevated CO_2 concentration on soybean gene expression. An analysis of growing and mature leaves. *Plant Physiology* **142,** 135–147.

Ainsworth E.A., Rogers A. & Leakey A.D.B. (2008) Targets for crop biotechnology in a future high-CO_2 and high-O_3 world. *Plant Physiology* **147,** 13–19.

Amthor J.S. (1998) Perspective on the relative insignificance of increasing atmospheric CO_2 concentration to crop yield. *Field Crops Research* **58,** 109–127.

Bernacchi C.J., Kimball B.A., Quarles D.R., Long S.P. & Ort D.R. (2007) Decreases in stomatal conductance of soybean under open-air elevation of [CO_2] are closely coupled with decreases in ecosystem evapotranspiration. *Plant Physiology* **143,** 134–144.

Bowes G. (1991) Growth at elevated CO_2: photosynthetic responses mediated through Rubisco. *Plant, Cell & Environment* **14,** 795–806.

Carter T.R., Jones R.N., Lu X., Bhadwal S., Conde C., Mearns L.O., O'Neill B.C., Rounsevell M.D.A. & Zurek M.B. (2007) New assessment methods and the characterisation of future conditions. In *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds M.L. Parry, O.F. Canziani, J.P. Palutikof, P.J. van der Linden & C.E. Hanson), pp. 133–171. Cambridge University Press, Cambridge, UK.

Cassman K.G., Dobermann A., Walters D.T. & Yang H. (2003) Meeting cereal demand while protecting natural resources and improving environmental quality. *Annual Review of Environment and Resources* **28,** 315–358.

Coats B. (2003) Global rice production. In *Rice Origin, History, Technology and Production* (eds C.W. Smith & R.H. Dilday), pp. 247–470. John Wiley & Sons, Hoboken, NJ, USA.

Cohen M.J. (2003) Food security: why do hunger and malnutrition persist in a world of plenty? In *Plants, Genes, and Crop Biotechnology* (eds M.J. Chrispeels & D.E. Sadava) 2nd edn, pp. 52–75. Jones and Bartlett Publishers, Sudbury, MA, USA.

Fichtner K., Quick W.P., Schultz E.-D., Mooney H.A., Rodermel S.R., Bogorad L. & Stitt M. (1993) Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with 'antisense' *rbcS*. *Planta* **190,** 1–9.

Gifford R.M. & Evans L.T. (1981) Photosynthesis, carbon partitioning, and yield. *Annual Review of Plant Physiology* **32,** 485–509.

Hansen J.W. & Jones J.W. (2000) Scaling up crop models for climate variability applications. *Agricultural Systems* **65,** 43–72.

Hansen J.W., Challinor A., Ines A., Wheeler T. & Moron V. (2006) Translating climate forecasts into agricultural terms: advance and challenges. *Climate Research* **33,** 27–41.

Heinicke J., Braun T., Burgassi P. & Italiano F. (2006) Gas flow anomalies in seismogenic zones in the Upper Tiber Valley, Central Italy. *Geophysical Journal International* **167,** 794–806.

Hendrey G.R. & Kimball B.A. (1994) The FACE program. *Agricultural and Forest Meteorology* **70,** 3–14.

Hendrey G.R. & Miglietta F. (2006) FACE technology: past, present and future. In *Managed Ecosystems and CO2. Case Studies, Processes, and Perspectives* (eds J. Nösberger, S.P. Long, R.J. Norby, M. Stitt, G.R. Hendrey & H. Blum), pp. 15–46. Springer, Berlin, Germany.

Hendrey G.R., Lewin K.F., Kolber Z. & Evans L.S. (1992) Controlled enrichment systems for experimental fumigation of plants in the field with sulfur dioxide. *Journal of Air & Waste Management Association* **42,** 1324–1327.

Hendrey G.R., Long S.P., McKee I.F. & Baker N.R. (1997) Can photosynthesis respond to short-term fluctuations in atmospheric carbon dioxide? *Photosynthesis Research* **51,** 179–184.

Hendrey G.R., Ellsworth D.S., Lewin K.F. & Nagy J. (1999) A free-air CO_2 enrichment system for exposing tall forest vegetation to elevated atmospheric CO_2. *Global Change Biology* **5,** 293–309.

Karnosky D.F., Werner H., Holopainen T., *et al.* (2007) Free-air exposure systems to scale up ozone research to mature trees. *Plant Biology* **9,** 181–190.

Kheshgi H.S. & Prince R.C. (2005) Sequestration of fermentation CO_2 from ethanol production. *Energy* **30,** 1865–1871.

Kimball B.A., Kobayashi K. & Bindi M. (2002) Responses of agricultural crops to free-air CO_2 enrichment. *Advances in Agronomy* **77,** 293–368.

Kimball B.A., Conley M.M., Wang S., Lin X., Luo C., Morgan J. & Smith D. (2008) Infrared heater arrays for warming ecosystem field plots. *Global Change Biology* **14,** 309–320.

Lanceras J.C., Pantuwan G., Jongdee B. & Toojinda T. (2004) Quantitative trait loci associated with drought tolerance at reproductive stage in rice. *Plant Physiology* **135,** 344–399.

Leakey A.D.B., Bernacchi C.J., Dohleman F.G., Ort D.R. & Long S.P. (2004) Will photosynthesis of maize (*Zea mays*) in the US Corn Belt increase in future [CO_2] rich atmospheres? An analysis of diurnal courses of CO_2 uptake under free-air concentration enrichment (FACE). *Global Change Biology* **10,** 951–962.

Leakey A.D.B., Bernacchi C.J., Ort D.R. & Long S.P. (2006a) Long term growth of soybean at elevated [CO_2] does not cause acclimation of stomatal conductance under fully open-air conditions. *Plant, Cell & Environment* **29,** 1794–1800.

Leakey A.D.B., Uribelarrea M., Ainsworth E.A., Naidu S.L., Rogers A., Ort D.R. & Long S.P. (2006b) Photosynthesis, productivity and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. *Plant Physiology* **140,** 779–790.

Lewicki J.L. & Oldenburg C.M. (2004) *Strategies for Detecting Hidden Geothermal Systems by Near-surface Gas Monitoring (LBNL-56895)*. Earth Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA.

Lewin K.F., Hendrey G.R. & Kolber Z. (1992) Brookhaven National Laboratory free-air carbon dioxide enrichment facility. *Critical Reviews in Plant Science* **11,** 135–141.

Lipfert F.W., Alexander Y., Hendrey G.R., Lewin K.F. & Nagy J. (1992) Performance of the BNL FACE gas injection system. *Critical Reviews in Plant Science* **11,** 135–141.

Long S.P., Ainsworth E.A., Rogers A. & Ort D.R. (2004) Rising atmospheric carbon dioxide: plants FACE the future. *Annual Review of Plant Biology* **55,** 591–628.

Long S.P., Ainsworth E.A., Leakey A.D.B., Nösberger J. & Ort D.R. (2006) Food for thought: lower-than-expected crop yield stimulation with rising CO_2 concentration. *Science* **312,** 1918–1921.

Manderscheid R. & Weigel H.J. (1997) Photosynthetic and growth responses of old and modern spring wheat cultivars to atmospheric CO_2 enrichment. *Agriculture, Ecosystems & Environment* **64,** 65–73.

Miglietta F., Lanini M., Bindi M. & Magliulo V. (1997) Free air CO_2 enrichment of potato (*Solanum tuberosum* L.): development, growth and yield. *Global Change Biology* **4,** 163–172.

Mikkelsen T.N., Beier C., Jonasson S., *et al.* (2008) Experimental design of multifactor climate change experiments with elevated CO_2, warming and drought: the CLIMATE project. *Functional Ecology* **22,** 185–195.

Morgan P.B., Bernacchi C.J., Ort D.R. & Long S.P. (2004a) An *in vivo* analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. *Plant Physiology* **135,** 2348–2357.

Morgan J.A., Pataki D.E. & Körner C., *et al.* (2004b) Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO_2. *Oecologia* **140,** 11–25.

Nowak R.S., Ellsworth D.S. & Smith S.D. (2004) Functional responses of plants to elevated atmospheric CO_2 – do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytologist* **162,** 253–280.

Ort D.R., Ainsworth E.A., Aldea M., *et al.* (2006) SoyFACE: the effects and interactions of elevated [CO2] and [O3] on soybean. In *Managed Ecosystems and CO2. Case Studies, Processes, and Perspectives* (eds J. Nösberger, S.P. Long, R.J. Norby, M. Stitt, G.R. Hendrey & H. Blum), pp. 71–85. Springer, Berlin, Germany.

Parry M.L., Rosenzweig C., Iglesias A., Livermore M. & Fischer G. (2004) Effects of climate change on global food production under SRES emissions and socio-economic scenarios. *Global Environmental Change* **14,** 53–67.

Porter J.R. & Semenov M.A. (2005) Crop responses to climate change. *Philosophical Transactions of the Royal Society B* **360,** 2021–2035.

Prioul J.-L., Quarrie S., Causse M. & de Vienne D. (1997) Dissecting complex physiological functions through the use of molecular quantitative genetics. *Journal of Experimental Botany* **48,** 1151–1163.

Schmidhuber J. & Tubiello F.N. (2007) Global food security under climate change. *Proceedings of the National Academy of Sciences of the United States of America* **104,** 19703–19708.

Solomon S., Qin D., Manning R.B., *et al.* (2007) Technical summary. In *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Annual Assessment Report of the Intergovernmental Panel on Climate Change* (eds S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor & H.L. Miller), p. 996. Cambridge University Press, Cambridge, UK/New York, NY, USA.

Tonsor S.J., Alonso-Blanco C. & Koornneef M. (2005) Gene function beyond the single trait: natural variation, gene effects and evolutionary ecology in *Arabidopsis thaliana*. *Plant, Cell & Environment* **28,** 2–20.

Tubiello F.N., Soussana J.-F. & Howden S.M. (2007) Crop and pasture response to climate change. *Proceedings of the National Academy of Sciences of the United States of America* **104,** 19686–19690.

USDA. (2007) *Grain: World Markets and Trade*. Foreign Agricultural Service/USDA Office of Global Analysis, Circular Series FG 12-07, http://www.fas.usda.gov/grain_arc.asp

Wall G.W., Brooks T.J., Adam R., *et al.* (2001) Elevated atmospheric CO_2 improved sorghum plant water status by ameliorating the adverse effects of drought. *New Phytologist* **152,** 231–248.

Yang H., Xu Z., Fan M., Gupta R., Slimane R.B., Bland A.E. & Wright I. (2008) Progress in carbon dioxide separation and capture: a review. *Journal of Environmental Sciences* **20,** 14–27.

Zeman F. (2007) Energy and material balance of CO_2 capture from ambient air. *Environmental Science & Technology* **41,** 7558–7563.

Zeman F.S. & Lackner K.S. (2004) Capturing carbon dioxide directly from the atmosphere. *World Resource Review* **16,** 157–172.

Ziska L.H., Morris C.F. & Goins E.W. (2004) Quantitative and qualitative evaluation of selected wheat varieties released since 1903 to increasing atmospheric carbon dioxide: can yield sensitivity to carbon dioxide be a factor in wheat performance? *Global Change Biology* **10,** 1810–1819.

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