24 FACE Value: Perspectives on the Future of Free-Air CO_2 Enrichment Studies

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24.1 The Value of FACE Experiments

Free-air CO_2 enrichment (FACE) studies are the ultimate test bed for hypotheses that seek to explain how plants respond to rising [CO_2]; and they provide the most realistic conditions for simulating the impact of future elevated (e)[CO_2] levels (see Chapter 2). FACE studies have many benefits over controlled environment and open-top chamber (OTC) experiments. FACE allows the investigation of an undisturbed ecosystem and does not modify the vegetation's interaction with light, temperature, wind, precipitation, pathogens and insects (Long et al. 2004). This, in combination with the large size of FACE plots, allows the integrated measurement of many plant and ecosystem processes simultaneously in the same plot, avoids many of the problems associated with edge effects prevalent in OTCs (Long et al. 2004), enables significantly more plant material to be harvested without compromising the experiment, and allows plants to be studied throughout their life cycle, including trees that have enough space to develop to canopy closure.

FACE experiments are not without their problems (see Chapter 2). In particular, there has been criticism that the application of a step increase in [CO_2] to ecosystems imposes a strong perturbation (Luo and Reynolds 1999; Newton et al. 2001). Luo and Reynolds (1999) modelled the effects of a step increase in [CO_2] and found that the demand for N caused by the additional carbon influx from the step increase is much greater than that caused by a gradual increase in [CO_2], resulting in an initial overestimation of carbon sequestration in experiments using a step increase in [CO_2]. This should be a minor problem in annual agro-ecosystems where plants have spent their whole life-cycle under FACE. However, in natural ecosystems, or perennial agro-ecosystems, a step increase in [CO_2] is more likely to have a significant effect. Continued support is crucial for many existing FACE studies if short-

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term disturbance effects are to be distinguished from long-lasting, permanent effects.

Another major problem for FACE experiments is that replication is limited by the costs of operation. FACE experiments typically have only three replicated treatment plots, and therefore, limited statistical sensitivity. As a result, it can be difficult to significantly detect small but physiologically important differences in the response of plants and ecosystems to rising [CO_2]. Deployment of FACE experiments is also limited by cost and the difficulty of deploying experiments in areas with poor infrastructure. This is more relevant for unmanaged ecosystems where fully replicated FACE experiments have yet to be deployed in tropical forests, and tropical savannas and grasslands, which together account for over half of the worlds net primary production (Prentice 2001).

24.2 What Have We Learnt From FACE?

Over 15 years of FACE experiments now provide enough data for quantitative integration of results and perhaps the most realistic predictions of how plants will respond to future atmospheric [CO_2]. Do the data from FACE experiments support early predictions of plant responses to e[CO_2]? This question has been addressed recently by a number of reviews (Ainsworth and Long 2005; Kimball et al. 2002; Long et al. 2004; Nowak et al. 2004). While FACE experiments confirmed many of the results from earlier chamber experiments, there were some surprising differences between fully open-air CO_2 enrichment studies and chamber experiments.

24.2.1 Photosynthesis and Aboveground Productivity

The response of photosynthesis to e[CO_2] has been reported for over 40 species at different FACE experiments (Ainsworth and Long 2005) and is discussed in more detail in Chapter 14. Stomatal conductance decreased 20 % under elevated [CO_2], but the ratio of intercellular [CO_2] to external [CO_2] did not change (Ainsworth and Long 2005). Apparent quantum yield of light-limited C_3 photosynthesis increased by 12 % under e[CO_2] (Ainsworth and Long, 2005) and C_3 light-saturated photosynthesis and diurnal carbon assimilation increased significantly at e[CO_2], providing the basis for increased aboveground dry matter production (DMP) and crop yield (Fig. 24.1). The increase in DMP and grain yield under e[CO_2] was less than the potential indicated by the increases in carbon uptake (Fig. 24.1). This suggests that there may be bottlenecks downstream of carbon acquisition that are limiting DMP, e.g. the supply of N and other nutrients. Leaf-area index (LAI) increased in trees and

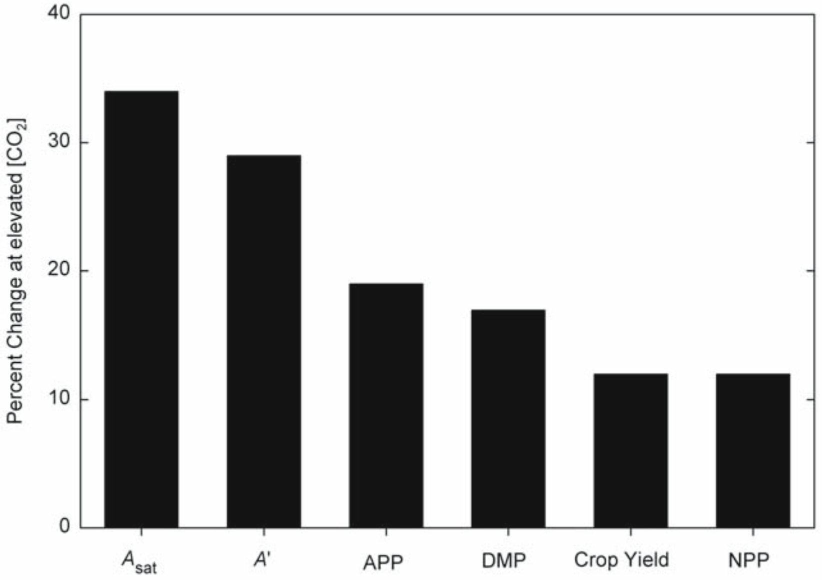

Fig. 24.1 Meta-analysis of FACE effects on light-saturated photosynthesis (A_{sat}), daily integral of carbon uptake (*A'*), aboveground primary production (*APP*), dry matter production (*DMP*), crop yield, and net primary production (*NPP*). A_{sat}, *A'*, DMP, and crop yield were adapted from Ainsworth and Long (2005) and APP and NPP were adapted from Nowak et al. (2004). In FACE experiments, the response of photosynthesis to e[CO_2] is greater than the response of dry matter production, which in turn is greater than the yield response

woody species, but differences between developing and closed canopy forests should be noted (see Chapters 10–13). LAI did not change in herbaceous species, which limited the stimulation of aboveground production and crop yield under FACE. Results from rice and wheat grown under FACE suggest that current productivity models (e.g. Izaurralde et al. 2003) are overestimating future yields and therefore providing overly optimistic world food production projections (Ainsworth and Long 2005).

24.2.2 Photosynthetic Acclimation

Reduced photosynthetic capacity (acclimation) of leaves grown under e[CO_2] has been shown to maintain a balance in N and other resources allocated to photosynthetic reactions (Drake et al. 1997; Rogers and Humphries 2000). In the field, foliar carbohydrates accumulated under e[CO_2], while N content, Rubisco content, and the maximum rate of carboxylation ($V_{c,max}$) declined (Fig. 24.2). The occurrence of photosynthetic down-regulation was both growth form- and environment-specific (Nowak et al. 2004). In FACE experi-

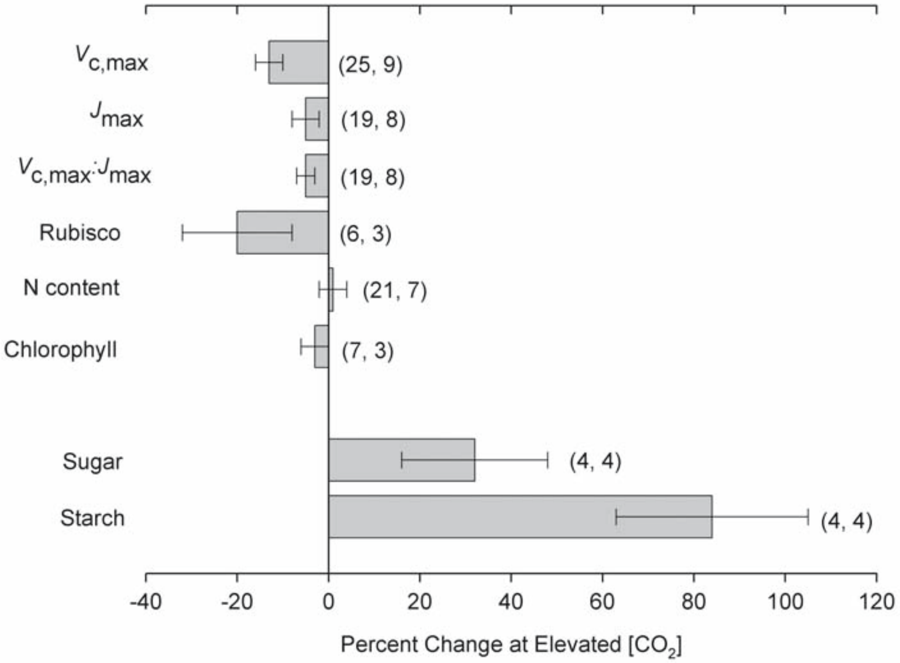

Fig. 24.2 Meta-analysis of acclimation of photosynthesis to e[CO_2] in FACE experiments, adapted from Ainsworth and Long (2005). The percent change in e[CO_2] ±95 % confidence intervals is shown. The numbers of species and FACE sites represented by each mean are shown in parentheses. Rubisco content, N content, chlorophyll content, sugar, and starch were all measured on a leaf area basis

ments, down-regulation of photosynthesis was observed under low-nutrient conditions, in old, but not young needles of evergreens, under cold temperatures late in the growing season, and in wet years, but not dry years (for reviews, see Ainsworth and Long 2005; Nowak et al. 2004). However, in relatively young trees grown under FACE, photosynthetic down-regulation did not occur (Nowak et al. 2004).

24.2.2.1 Response of Different Functional Groups

Analysis of different functional groups has revealed some generalities, but results do not always fit anticipated patterns (Nowak et al. 2004). Generally, C_4 species were less responsive than C_3 species to the increases in [CO_2] provided by FACE experiments (Ainsworth and Long 2005). On average, light-saturated photosynthesis increased marginally (10 %), while diurnal carbon assimilation and dry matter production did not change in C_4 species exposed to e[CO_2] (Ainsworth and Long 2005). However, when C_4 plants were waterstressed, improved water status from reduced stomatal conductance led to a significant stimulation of photosynthesis (Leakey et al. 2004). Clearly, it is the interaction between species and the environment that determines responsiveness to e[CO_2].

To date, trees have been more responsive to e[CO_2] in FACE studies than other functional groups, namely grasses, forbs and legumes (Ainsworth and Long 2005; Nowak et al. 2004). For 12 tree species measured at five different FACE sites, photosynthetic CO_2 uptake increased by 47 %, branch number increased by 25 %, stem diameter was 9 % larger, and LAI increased by 21 % (Ainsworth and Long 2005). Trees also showed a large stimulation in dry matter production and cotton, a woody crop, showed significant and large increases in yield (Ainsworth and Long 2005). This observation is contrary to expectations from earlier studies; however, most of the trees grown under FACE have been young and rapidly growing. Our understanding of the response of mature, closed-canopy forests to e[CO_2] is improving (see Chapter 11), but long-term observations will be required to resolve many of the most basic questions about forested ecosystems. Given the importance of old-growth forests as a carbon pool and potential sink (Carey et al. 2001; Schulze et al. 2000), the value of a future, large, well replicated, long-term FACE experiment in an old-growth forest is clear.

24.2.2.2 Belowground Responses

Effect of C:N on Decomposition. The observation of lower N concentrations in plant tissue grown at e[CO_2] (Cotrufo et al. 1998) led to the hypothesis that the decomposition of plant leaf litter with a higher C:N ratio would be slower at e[CO_2], and possibly limit ecosystem productivity in a CO_2-enriched world (Strain and Bazzaz 1983). Although some experimental evidence has been found for this, the majority of studies failed to support this initial hypothesis (Norby and Cotrufo 1998). Differences in C:N ratios in green tissue were often absent or insignificant when leaves were allowed to become senescent in situ (Norby et al. 2001), but recent studies in the AspenFACE and in the POP/EUROFACE experiments are reporting increased C:N ratios in litter from e[CO_2] plots (see Chapters 10 and 12). However, potential species shifts caused by growth at e[CO_2] may play a greater role in changing the rates of litter decomposition and subsequent nutrient cycling than the direct effects of e[CO_2] on tissue quality (Allard 2004; Kammann et al. 2005).

Primary Productivity. Nowak et al. (2004) recently reviewed belowground primary productivity (BPP) in FACE experiments located on forest, bog, grassland, and desert sites. BPP increased on average by 32 % in e[CO_2] and varied from a 70 % increase in forests to a 7 % decrease in desert systems. However, these differences in ecosystem responses to e[CO_2] were not significant, largely due to the difficulty in measuring BPP accurately (Nowak et al. 2004). New methods for quantifying BPP are urgently needed.

Carbon and Nitrogen Sequestration. In their review, Zak et al. (2000) reported large increases and decreases in soil microbial biomass and microbial C and N in response to growth at e[CO_2]. However, total microbial C and N pools were unaltered and it was changes in microbial community composition and function that were important (Montealegre et al. 2000; Lukac et al. 2003). Groenigen et al. (Chapter 21) reviewed the effects of e[CO_2] on soil C and soil N. Briefly, soil C increased in FACE systems in an N-dependent manner (Fig. 24.3). Under high N fertilization conditions, there was a 9 % increase in soil C, but under medium and low N fertilization, there was no significant change in soil C. Soil N increased slightly under FACE (2.8 %) and the C:N ratio increased in experiments with soil disturbance (Fig. 24.3).

Evidence for N Limitation Feedback on Carbon Acquisition. The above findings suggest that N availability did not decrease, nor did it diminish the response of vegetation to e[CO_2]. Further support of this claim comes from the 10-year Swiss FACE experiment, where photosynthetic stimulation did not change over the course of that experiment, suggesting that carbon acquistion by *Lolium perenne* monocultures did not become N-limited (Ainsworth et al. 2003). In contrast, Oren et al. (2001) attributed the loss of the initial stimulation in the annual carbon increment of *Pinus taeda* grown at e[CO_2] in the Duke Forest prototype experiment to a possible nutrient limitation. Following application of fertilizer, the CO_2-induced biomass carbon increment returned to the levels observed at the start of the experiment, suggesting that N limita-

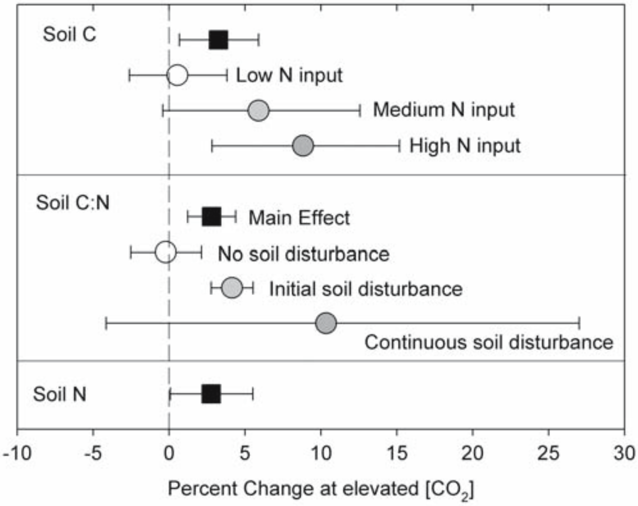

Fig. 24.3 A meta-analysis of FACE effects on soil C, soil N, and the ratio of C:N (data provided by Kees Jan van Groenigen and Marie Anne De Graaff). The percent change at e[CO_2] ±95 % confidence intervals is shown (n=57 for soil C, n=32 for soil C:N, n=39 for soil N)

tions had constrained the response to e[CO_2] (Oren et al. 2001). In the adjacent replicated Duke Forest FACE experiment, net primary production (NPP) was correlated with net N mineralization rate but was also related to interannual variation in rainfall and degree-days, (see Chapter 11). Whilst an insufficient N supply may limit the response of plants to elevated [CO_2], more often than not, in managed ecosystems, N limitation of carbon acquisition has not resulted from a step change in [CO_2].

24.3 What Is Missing From Current FACE Research and What Are the Gaps in Understanding?

24.3.1 Additional Treatments

The predicted increase in [CO_2] from 540 ppm to 970 ppm by 2100 (Prentice 2001) will not occur in isolation and the importance of studying the effect of rising [CO_2] in conjunction with other predicted global change is clear. Of particular importance are the predicted 62 % increase in tropospheric ozone concentration [O_3] (Prather and Ehhalt 2001), the predicted increase of between 1.4 °C to 5.8 °C in mean global temperature, and less certain predicted regional variations in precipitation (Cubasch and Meehl 2001). Water supply was added as a split-plot treatment as part of the FACE experiment at the Maricopa Agricultural Research Center, Ariz., and a rainfall displacement study was piloted at the SoyFACE experiment (Kimball et al. 1995; ADB Leakey, personal communication). Two FACE facilities currently include interactions with e[O_3] (Karnosky et al. 1999; Morgan et al. 2004); and free-air temperature increase was used to elevate canopy temperature as part of the SwissFACE project (Nijs et al. 1996). Given that the response to the combination of two treatments is not always predictable from the response of individually applied treatments, it is clear that more field studies that include interactions between predicted global change variables are needed. This is particularly important because current process-based terrestrial models that are used to model terrestrial carbon cycling incorporate interactions between changes in [CO_2] and climate that have yet to be validated in fully open-air field experiments (Prentice 2001); and of particular importance is the inclusion of physiological acclimation. Hanson et al. (2005) demonstrated the importance of including physiological acclimation in C-cycle models. When included, estimates of annual net ecosystem exchange in an upland-oak forest increased from a 29 % reduction under a multiple global change scenario to a 20 % stimulation. Nesting small drought or temperature treatments within larger CO_2 enrichment plots does not provide the best prediction of the response of plants and ecosystems to multiple atmospheric and climatic changes. Larger experiments with fully replicated designs are required.

The value of FACE experiments is their ability to mimic the predicted increase in [CO_2] and [O_3] in as realistic an environment as possible. Care must be taken to ensure that future experiments including interactions with precipitation, and in particular temperature, are designed to reproduce, as closely as possible, the predicted global change scenarios and that they are largely free of artifacts. If this is not done, the value of applying these treatments in the field is significantly reduced.

Simulating global warming in field studies is difficult, and to date, OTCs may offer the best platform for field temperature increases, but as discussed above, OTCs have significant drawbacks. Given what we already know about the response of plants to temperature (Long and Woodward 1988), it is clear that a fully open-air experimental design should include warming of the plant and soil. However, available technologies, namely the use of infrared lamps to heat plant canopies (Nijs et al. 1996) and alternative treatments that warm plant roots (Melillo et al. 2002), have problems. With infrared lamps, the canopy is not warmed uniformly. With low-stature grasses this problem is less serious, but as LAI increases, preferential warming of the upper canopy becomes a problem. Infrared lamps warm and dry the air, increasing the vapor pressure deficit. Global warming is not predicted to be associated with drier air, nor will global temperature increases occur through an exclusive warming of the soil. Even defining the nature of the temperature treatment is challenging. Over the past 50 years, warming on land has been significantly more pronounced at night and mid-to high-northern latitude winters have become warmer, whilst summers have shown little change (Cubasch and Meehl 2001). In addition to diurnal and seasonal patterns, the frequency, timing and duration of temperature extremes will also significantly impact plant responses to global warming (Morison and Lawlor 1999). It is possible that simple ambient +2 °C-type treatments may fail to provide the information we need to predict the response of our ecosystems to future global change scenarios.

24.3.2 Future Challenges

To date, FACE studies have been limited to just a few species, in managed ecosystems, and only one or two varieties of each cultivar. We therefore have little understanding of the variation in germplasm responses to e[CO_2]. Studies have been conducted mostly in temperate ecosystems and these studies have usually only taken place at one location for ecosystems that cover many degrees of latitude. Aboveground research has focused on the response of recently matured leaves at the top of the canopy, but much less is known about developing or senescing foliage. However, on the whole, observation of aboveground plant physiological processes in FACE experiments is well advanced and our future challenge will be to provide mechanistic explanations for the commonly observed physiological responses to growth at e[CO_2].

In order to understand the mechanisms that underlie the response of plants and ecosystems to global change, it will be necessary to identify the mechanistic links between levels of biological organization, from changes in gene expression profiles to physiological responses and ultimately to changes in crop yield and the structure and function of ecosystems. This will require large, multidisciplinary, research teams capable of examining responses at macromolecular to ecosystem scales.

Plants and ecosystems sense and interact with rising [CO_2] through increased photosynthesis and reduced transpiration (Long et al. 2004); and, given that the [CO_2] within the soil is 1–3 orders of magnitude greater than atmospheric [CO_2], it is extremely unlikely that soil microorganisms will respond directly to a ca. 200 ppm increase in atmospheric [CO_2]. However, changes in nutrient cycling via soil microbial, fungal, and faunal communities have the potential to modulate leaf-level responses to e[CO_2], so understanding subsurface processes is an essential part of a full and complete understanding of the response of plants and ecosystems to rising [CO_2]. Of particular relevance to managed ecosystems is the need to understand how growth at e[CO_2] may impact soil fertility and C sequestration.

Many important questions about how soil processes, particularly C and N cycling, will respond to rising [CO_2] remain unanswered and continue to be a challenge. This challenge is in part due to the problem of understanding and quantifying soil processes. To meet this challenge, new approaches are needed to overcome the difficulties associated with the tremendous heterogeneity of soil and the problem of quantifying small and slow acting processes relative to extremely large C and N pool sizes. Several key challenges remain.

24.3.3 What Is the Fate of C Partitioned Belowground?

Closing the carbon budget has been a focus of FACE experiments; and the fate of carbon partitioned belowground has proved a major challenge. A key piece of this puzzle is soil respiration; the magnitude and direction of the response of soil respiration to e[CO_2] varies with soil type, climate, seasonality, stand development, and species composition (King et al. 2004; Zak et al. 2000). The contribution of deep roots may be a small, unaccounted-for carbon sink in deeper soils under e[CO_2] (Lukac et al. 2003; Marchi et al. 2004); and a proportional increase in deep roots under e[CO_2] may have several impacts on soil organic matter (SOM) degradation and trace gas production. Indeed, the question of how much of the C partitioned belowground ultimately remains in the soil as SOM under e[CO_2] is an important one for ecologists and policy makers (see Chapter 21)

24.3.3.1 N Cycling

Early predictions that increased N immobilization would limit the response of plants to e[CO_2] have been tempered by whole-ecosystem studies (Norby et al. 2001; Strain and Bazzaz 1983). Symbiotic and free N fixation may increase at e[CO_2] but the impact of a possible P limitation on N fixation is not certain. To date, experiments have been conducted in plants grown in pots in controlled environments but the response of field-grown N-fixers to P limitation at e[CO_2] is unknown (Almeida et al. 1999, 2000; Niklaus and Körner 2004). The response of net nitrification rates to e[CO_2] is unclear, with both increases and decreases reported (Barnard et al. 2004; Ross et al. 2000). Gross N mineralization rates were either unchanged or increases were not significant under FACE (Finzi et al. 2002; Richter et al. 2003). It is unclear if increases in gross N mineralization, combined with rising N-use efficiency (Drake et al. 1997), will meet the rising N demand under e[CO_2] and sustain increases in NPP.

24.3.3.2 Soil Faunal Food Webs and Soil Structure

How will root life span, architecture, and exudation change at e[CO_2] and impact soil food webs? Root colonization by ectomycorrhizal and arbuscular mycorrhizal fungi usually increases under e[CO_2] (Lukac et al. 2003; Rillig et al. 2001). Arbuscular mycorrhizal fungi produce glomalin, which stabilizes soil aggregates (Wright and Anderson 2000). Since glomalin concentration is often increased at e[CO_2] (Rillig et al. 2001) and declines in water repellence have been also been reported (Newton et al. 2004), it is clear that the physical property of the soil could change at e[CO_2]. Such changes may impact erosion, fertility, protection of organic matter in aggregates, and hence C sequestration, but little is currently known. Soil faunal community services (e.g. earthworms) play a crucial role in soil fertility and nutrient cycling, but the responses of soil fauna to e[CO_2] are inconsistent (Coûteaux and Bolger 2000). Effects often reflect increases in net BPP, e.g. root-feeding nematode species increased in abundance at e[CO_2] (Yeates et al. 2003). This is particularly important for managed ecosystems, since many of these species are pests/pathogens of economical importance. Also, the effect that e[CO_2]-induced changes in soil faunal food webs may have on the performance and subsequent decomposition of herbicides, fungicides, and pesticides is unknown.

24.3.3.3 Trace Gases

The effect of e[CO_2] on fluxes of the greenhouse gases nitrous oxide (N_2O) and methane (CH_4) have rarely been investigated in FACE experiments.

Methane emissions from wetlands are usually stimulated at e[CO_2] (Dacey et al. 1994; Inubushi et al. 2003; Saarnio et al. 2000). On the few occasions they were measured, CH_4 oxidation rates (uptake) were mostly reduced at e[CO_2] (Ineson et al. 1998; Phillips et al. 2001a). Overall, there is evidence for a positive feedback of e[CO_2] on CH_4 fluxes, but this relationship is poorly understood. Nitrous oxide emissions increased at e[CO_2] due to denitrification when nitrate was readily available (Baggs et al. 2003b; Ineson et al. 1998). Nitrous oxide fluxes due to nitrification and denitrification were also dependant on soil moisture content and showed seasonal variation (Phillips et al. 2001b). Clearly, it is important to calculate annual budgets for N_2O emissions to understand whether the overall feedback of e[CO_2] on N_2O emissions will be positive or negative. This is especially important for managed ecosystems where N fertilization and irrigation are part of management practices.

24.4 Technologies for Future FACE Science

24.4.1 The Use of Stable Isotopes

The isotopic signatures of carbon and nitrogen pools provide the means of tracking the transformation of carbon and nitrogen as they flow through plant and soil compartments and the possibility of separately investigating individual soil processes. Two uses of stable isotope technology have emerged, one using small, mostly natural ^{13}C and ^{15}N approaches and the other using high-enrichment, pulse-chase techniques by applying ^{15}N fertilizer, or a $^{13}CO_2$ or $^{14}CO_2$ pulse that is incorporated into the plant–soil system by photosynthesis (Pendall 2002).

The supply of CO_2 used for fumigation in FACE experiments is usually ^{13}C-depleted. This provides the means to distinguish between carbon pools formed prior to CO_2 enrichment and those formed post-fumigation (Hungate et al. 1997). Unfortunately, these measurements are restricted to the elevated CO_2 plots, but root in-growth cores containing soil with an isotopically distinct ^{13}C signature have been used to allow the quantification of C inputs in the control, as well as elevated CO_2 plots (van Kessel et al. 2000). Future FACE experiments on C_3 plants could perhaps be sited on long-term C_4 soils (e.g. *Zea mays* or *Sorghum* fields). Such an approach has been used successfully to quantify carbon inputs due to forest re-growth on an afforested maize field (Del Galdo et al. 2003). However, the advantages afforded by such an approach must be weighed against growing C_3 plants on non-natural or unsuitable soils where the responses may differ from those occurring in their natural environment.

Soil microbial biomass discriminates against ^{15}N (Robinson 2001) and therefore the ^{15}N abundance in plant material may offer a promising tool to better understand plant–microbial interactions and gross N transformation processes at e[CO_2]. Observed enrichment of ^{15}N in plant material grown at e[CO_2] may indicate a possible increase in microbial activity and an improved root–microbial exploration of old, recalcitrant N pools (Billings et al. 2004). A recent synthesis of $\delta^{15}N$ depletion in foliage grown at e[CO_2], mostly in FACE experiments, concluded that e[CO_2] strongly influenced the N cycle but it was unclear where in the N cycle that the effects of e[CO_2] were manifested (BassiriRad et al. 2003).

In recent years, isotopic pulse labeling of a certain C and N fraction with a high isotopic enrichment of up to 99 % above natural abundance has become a valuable tool in the investigation of C and N cycling in ecosystems. For example, Staddon et al. (2003) used a ^{14}C pulse-labelling approach to determine that the extraradical hyphae of arbuscular mycorrhizal fungi live on average 5–6 days and hence comprise a large, but rapidly turned-over C pool in the soil C cycle. By use of ^{13}C pulse-chase techniques, the short-term gross flow of ^{13}C through various soil C pools, processes and soil organisms might readily be determined in FACE experiments (Ostle et al. 2000; Radajewski et al. 2000). Likewise, labelling with ^{15}N applied as fertilizer to the soil enables N transformation and sequestration processes to be followed in various soil compartments (Baggs et al. 2003a; Richter et al. 2003). The combination of ^{15}N labelling and tracing techniques with new process-based modelling approaches (Müller et al. 2004) may provide new insights into N transformation processes in soils at e[CO_2].

24.4.2 Genomic Technologies and Tools in FACE

Advances in ‘omics’ technologies have produced an explosion of biological information at the level of transcripts, proteins, protein modifications, and metabolites.

High-throughput technologies allow examination of thousands of genes, proteins and metabolites in parallel (Aharoni and Vorst 2002; Kersten et al. 2002; Kopka et al. 2004; Weckwerth 2003). Systems biology uses understanding of molecular networks and biological modules from functional genomics approaches to comprehend the basis of complex phenotypes (Blanchard 2004; Kitano 2002). Predictive models of biological systems that incorporate biochemical and genetic data are a goal of systems biology and analogous to the approach of global change biology, where advances in technology have spurred realistic experimental platforms for data collection, which provide information for predictive computational models. The challenge is to incorporate large data sets on spatial and temporal scales of genes, metabolites and proteins into models that enable explanation of plant responses to predicted global change events. One step towards this goal will be the development of diagnostic markers for given physiological conditions, e.g. N limitation or oxidative stress. These markers would need to include a temporal dimension to separate short-term and long-term responses. Early steps toward identifying robust markers for C starvation and N assimilation have been made (Foyer et al. 2003; Gibon et al. 2004) and suggest that the identification of diagnostic markers for other physiological processes will also be possible.

The 2004 special issue of *Field Crops Research* (vol 90, issue 1) highlighted the subject area: *Linking functional genomics with physiology for global change research*. Only one case-study from a FACE experiment (*Arabidopsis* grown at SoyFACE) was included in this special issue; and it discussed some of the challenges of applying transcript analysis to field-grown material (Miyazaki et al. 2004). A general conclusion from the study was that results revealed a snapshot of time- and weather-dependent transcripts, superimposed on responses to e[CO_2] and e[O_3], and repeated profiles of expression would be needed to determine FACE-specific patterns (Miyazaki et al. 2004). This study warned that application of functional genomics to global change science will certainly be challenging, but advances in genomic technologies for other species (including poplar, soybean, maize, and rice) will aid in merging the fields. Integration of molecular data into models should improve our predictions of plant responses to global change.

24.5 A Potential Problem for Long-Running FACE Experiments?

It is clear that, in order to answer many of the questions associated with fundamental belowground processes, operation of FACE experiments must be continued for a significant length of time. This is particularly important for experiments where species composition changes may be slow to appear, where a step increase in [CO_2] has been applied to a mature ecosystem, and in experiments on forest systems where it may take several years to reach canopy closure. Currently the longest-running CO_2 enrichment experiment is the OTC experiment on *Scirpus olneyi* (Rasse et al. 2005) that began CO_2 fumigation in 1987. Due to seasonal variation in meteorological conditions, the effect of e[CO_2] over time is often assessed by comparing the ratio of a response at e[CO_2] to the response of the c[CO_2] control; and this could lead to a misinterpretation of the CO_2 response. Consider a hypothetical 25-year FACE experiment running from 1990 to 2015 with a treatment [CO_2] set point of 550 ppm. Over the course of the experiment the control [CO_2] would have risen from 354 ppm to 392 ppm (Prentice 2001), whilst the treatment [CO_2] would have remained unchanged at 550 ppm. If we assume that the relationship between net CO_2 assimilation (A) and intercellular [CO_2] (c_i) remained constant over the course of the experiment and we use the equations of Farquhar et al. (1980) to calculate photosynthetic stimulation based on three categories of

Table 24.1 Reduction in stimulation of *A* in FACE plots relative to current [CO_2] control plots due to rising [CO_2] over a hypothetical 25-year FACE experiment starting in 1990 when the [CO_2] was 354 ppm. The increase in [CO_2] over the course of this hypothetical experiment is based on the IPCC-predicted increases of 1.5 mmol mol^{-1} $year^{-1}$ (Prentice et al. 2001)

	Modeled assimilation[a] ($\mu mol\ m^{-2}\ s^{-1}$)		
[CO_2] (ppm)	C_3 crop	Hardwood	Conifer
354	18.03	8.89	4.21
392	19.84	9.83	4.72
550	22.19	13.30	5.43
588	22.58	14.03	5.54
Year stimulation modeled, with [CO_2] (ppm)	Percent stimulation in *A* at elevated [CO_2]		
	C_3 crop	Hardwood	Conifer
1990	33	50	29
2015 (set point, 550)	21	35	15
2015 (ambient + 196)	23	43	17

[a] *A* was modeled using the equations of Farquhar et al. (1980). PPI = 1400 $\mu mol\ m^{-2}\ s^{-1}$, temperature = 25 °C, $c_i/c_a = 0.7$, RH = 90 %. Values for $V_{c,max}$ and J_{max} were taken from Wullschleger (1993). $V_{c,max}$ = 90, 47 and 25 $\mu mol\ m^{-2}\ s^{-1}$ and J_{max} = 171, 104 and 40 $\mu mol\ m^{-2}\ s^{-1}$ for a C_3 crop, hardwood, and conifer, respectively

plant: C_3 crop, hardwood, and conifer (Wullschleger 1993), the mean relative stimulation in *A* across these three types of vegetation would be 37 % at the beginning of the experiment and only 24 % at the end of the experiment (Table 24.1). This problem would not be solved by adopting an ambient +196 ppm fumigation protocol rather than a set point of 550 ppm because the relationship between photosynthesis and [CO_2] is not linear. Due to the shape of the A/c_i plot, long-term FACE experiments over hardwood forests may, to some degree, be able to ameliorate this confounding factor by the use of an ambient +196 ppm fumigation protocol (Table 24.1). Since almost all responses of plants and ecosystems to rising [CO_2] occur downstream of photosynthesis, there is potential to misinterpret the consequences of an apparent reduction in carbon acquisition in long-running experiments.

24.6 Conclusion

Free-air CO_2 enrichment studies have been a valuable tool for the investigation of plant and ecosystem responses to rising CO_2 levels. The challenges for the next phase of FACE research are clear.

- Multidisciplinary teams of investigators must take advantage of emerging technologies to significantly increase our mechanistic understanding of the responses that FACE experiments have confirmed will take place during the next century.
- If we seek the ability to predict and understand how our managed, and natural, ecosystems will respond to the predicted multiple and concurrent changes in our environment, more interactions with other global change factors must be included in future experiments. To meet these challenges, future FACE experiments will need to be larger to accommodate multiple environmental changes.

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