Opinion

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Challenges in elevated CO_2 experiments on forests

ESF-Forest FACE Group

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Current forest Free Air CO_2 Enrichment (FACE) experiments are reaching completion. Therefore, it is time to define the scientific goals and priorities of future experimental facilities. In this opinion article, we discuss the following three overarching issues (i) What are the most urgent scientific questions and how can they be addressed? (ii) What forest ecosystems should be investigated? (iii) Which other climate change factors should be coupled with elevated CO_2 concentrations in future experiments to better predict the effects of climate change? Plantations and natural forests can have conflicting purposes for high productivity and environmental protection. However, in both cases the assessment of carbon balance and how this will be affected by elevated CO_2 concentrations and the interacting climate change factors is the most pressing priority for future experiments.

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Forest ecosystems under climate change

Carbon dioxide (CO_2) is the most important greenhouse gas emitted from anthropogenic sources both in terms of amount and effects on climate. Despite some efforts aimed at limiting anthropogenic CO_2 release, the annual growth rate in CO_2 concentration was larger during recent years (1995–2005, average: 1.9 ppm/year) than it has been since the beginning of continuous direct atmospheric measurements (1960–2005, average: 1.4 ppm/year) [1]. More than 85% of all biomass carbon (C) is tied up in forests and the tropical forests alone stock 340 gigatons of C (i.e. 52% of all biomass-C) [2]. A CO_2 fertilization effect (i.e. a stimulation of productivity under increasing CO_2 levels in forest ecosystems) has been demonstrated in field experiments [3,4], and the potential role of forests in ameliorating climate change by removing CO_2 from the atmosphere has been demonstrated in models [1]. However, the ability of the biosphere to reduce atmospheric CO_2 concentrations seems to be weakening [5]. Recognizing the importance

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of forests in the global carbon cycle and climate change, the XVII International Botanical Congress in 2005 promulgated one specific resolution on global change, emphasizing the need for spatiotemporally large-scale experiments on plant (particularly tree) responses to elevated CO_2 concentrations and calling for international collaboration to bring experiments such as Free Air CO_2 Enrichment (FACE) to fruition. Although large-scale experiments examining the path of carbon through forest ecosystems that already existed in 2005 [6–10] have continued and most have now been or soon will be completed, no new large-scale forest experiments have been initiated since the 2005 report was published. FACE is the best technique available for a large-scale investigation of ecosystem responses to elevated CO_2. Originally designed as an open-air fumigation technique for air pollution studies in crop systems [11], FACE was adapted for CO_2 releases and scaled-up for application in forests [6]. This allowed long-term and whole-ecosystem studies that were not possible with previous indoor or field chamber experiments with tree seedlings or young saplings. Further modifications of the FACE approach also allowed experiments to be conducted in mature forest ecosystems and measurements of elevated CO_2 concentrations with elevated ozone (O_3) concentrations to be combined, another major factor of global change [7,8,12]. Because most current FACE sites in forest ecosystems are now coming to completion (http://www.nature.com/news/2008/081118/full/456289a.html), it is time to reflect upon the most relevant questions and associated challenges for the next generation of experiments on elevated CO_2.

Questions for the next generation experiments

We believe that future forest FACE experiments should focus on the effect of the increase in CO_2 concentration on global carbon cycling of forests. This issue has crucial implications given the environmental role of forests in sequestering carbon from the atmosphere but also because it is related to the productivity of the different forest ecosystems. Unfortunately, most of the questions related to carbon cycling have a time frame of 50–100 years; thus, the purpose of the experiments is to quantify fundamental responses that must be represented in ecosystem and global models. The current FACE experiments might not have run long enough to reveal negative feedback owing to progressive nutrient limitation (i.e. nitrogen) [13], and the separation of direct CO_2 effects (via photosynthesis) from indirect CO_2 effects driven by water savings (via stomata) proved to be very difficult [14]. Thus, a better understanding is required on the interactions between elevated CO_2 and nitrogen over long periods of time. Also, recognizing that increases in atmospheric CO_2 will be accompanied by changes in precipitation, temperature and O_3 means that we should consider how these factors affect the response of forest ecosystems to elevated CO_2. The opportunities and challenges for addressing these overarching questions are presented below.

General priorities for future research on forests

To obtain an estimate of plant growth potential and carbon sequestration with rising CO_2, it is necessary that future experiments provide a complete carbon budget for each ecosystem. In particular, the primary objective is to identify the allocation of extra carbon taken up as many of the experiments carried out to date have shown a certain amount of "missing carbon" which can be ascribed to an underestimation of belowground allocation [15]. Soil not only represents an unknown factor with regard to its capacity to act as a carbon sink but contains processes that can directly impact on the CO_2 response [16]. Thus, it is crucial that the carbon budget is studied in large multifactorial FACE experiments, with specific emphasis on soil nutrition coupled with elevated CO_2 concentrations, because the responses of these two interacting factors have been variable in previous experiments [17–19]. Because disturbance of soils (e.g. by planting, grading) interrupts the intimate plant–rhizosphere coupling, future experiments should aim for test conditions in which the carbon and nutrient cycles are in a quasi steady state at the onset of the elevated CO_2 treatment [14]. The strong relationships between plant and soil further reinforce the need for ecosystem approaches in future experiments and the need for experiments designed to last for up to two decades.

Future FACE experiments should combine regimes with precipitation and temperature manipulation together with elevated CO_2 concentrations, as is already done in shrubland and grassland ecosystems [20–22]. Furthermore, current experiments have shown that ozone can offset or negate the stimulatory CO_2 effects on above ground vegetation [23]. At the same time, determining how ozone interacts with increasing CO_2 concentrations at below ground level will be critical for accurately predicting future forest growth and carbon allocation.

We suggest the use of natural thermal gradients between test sites within a given region when costs of manipulating the various factors influencing the global climate (i.e. warming, drought or O_3) are prohibitive. This issue is particularly relevant because the effects of these factors can be far from additive, making the impacts of the combined factors difficult to predict [24]. By contrast, this approach requires site homogeneity in soil substrate, forest stand composition, genetic uniformity and a pretreatment analysis that accounts for much of the variability of the selected ecosystems.

The last priority we would like to list here is the need to understand the climate–ecosystem feedback of climate change [1]. Alterations in greenhouse gas fluxes in biogenic volatile organic compounds emitted and in albedo and surface energy balance represent the most important unknown climate–ecosystem feedback that must be understood in the future generation of large-scale experiments to enable efficacious climate model projections.

Priorities for natural or semi-natural forests

Genetic and species biodiversity are the primary issues to be investigated in the next generation of experiments with natural ecosystems under elevated CO_2 conditions (Figure 1). This will require studies of plots containing a natural diversity of plants and other organisms because the response to elevated CO_2 concentrations of a species depends on the neighboring species. Mono-specific plant stands might show responses different from mixed plant

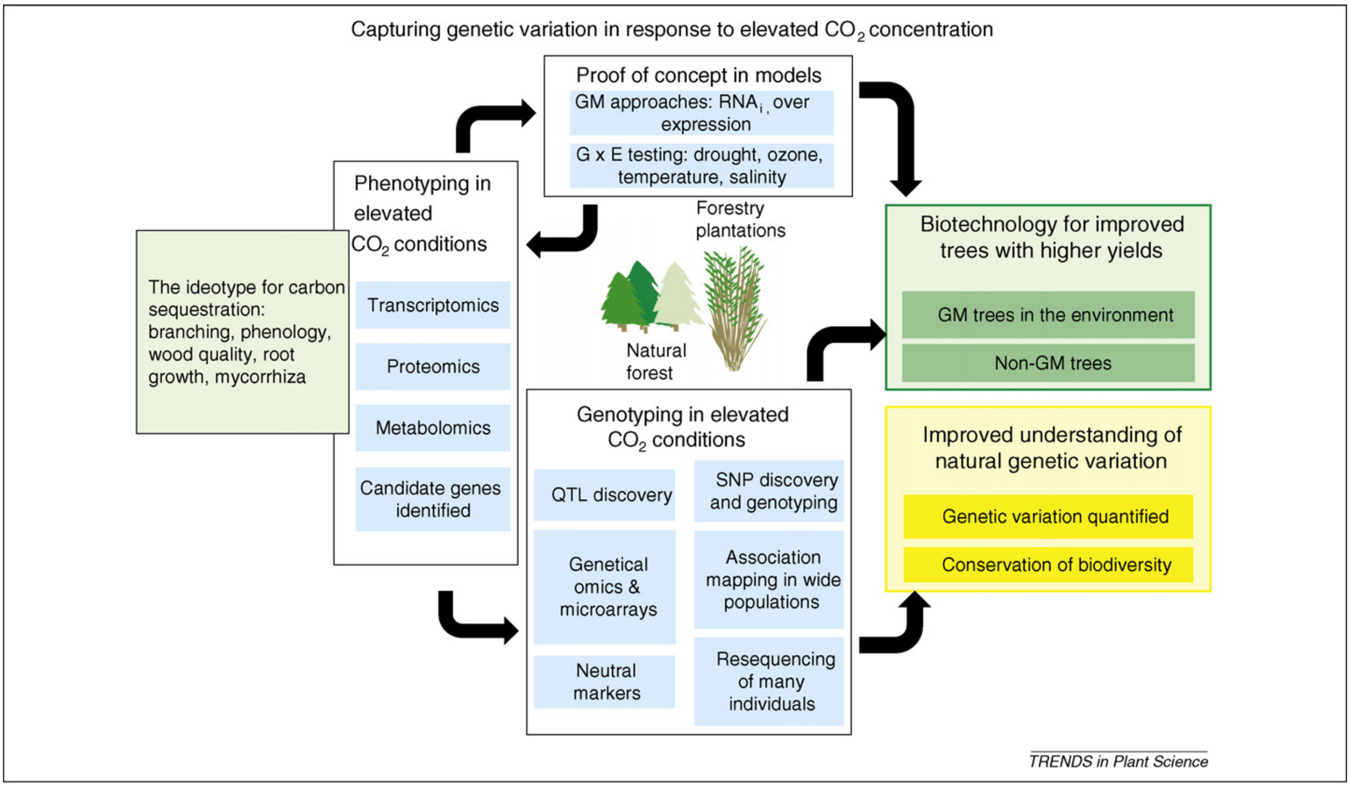

Figure 1. Systems biology approaches to provide insight into quantifying forest ecosystem response to elevated CO_2 conditions and understanding the natural genetic variation that might exist in response to CO_2. Using the latest technologies in functional omics, including transcriptomics, proteomics and metabolomics, should produce a phenotypic fingerprint underpinning the ideotype needed for fast growth and carbon sequestration. Proof of concept will require reverse genetic approaches such as RNAi and overexpression studies and this could result in genetically modified (GM) trees with enhanced traits of interest or non-GM trees improved by molecular breeding (green boxes). Second generation sequencing technologies can be applied to the study of natural genetic variation in response to increasing CO_2 enabling appropriate future conservation strategies (yellow boxes).

stands; moreover, elevated CO_2 concentrations have been shown to modify competition among plant species [18,25]. A major advance in our knowledge would come from studying mature and diverse forest stands with similar standing biomass and nutrient cycles for a sufficient time to measure ecosystem level changes. One major problem is species specific responses, the causes (mechanisms) of which are commonly not understood.

We recommend the application of new genomic tools such as microarray and high-throughput protein and metabolite screening (Figure 1) for the quantification even of small intra- as well as inter-specific differences in signal chains related to specific response traits [26]. Assessing and quantifying natural genetic variation at the genome level through neutral and single nucleotide polymorphism based molecular tags will also be an important future target for forest biologists to guarantee the sustainability of natural ecosystems and to cope with climate change.

Priorities for forest plantations

The human need for renewable energy sources has focused global interest in increasing the areas occupied by high-productivity forest plantations such as short rotation forestry (SRF). Advances have recently been made in the use of 'omic' techniques to understand the mechanistic basis of plant responses to elevated CO_2 concentrations [27,28].

We need to screen a very wide range of germplasm for the most common species of *Populus*, *Salix*, *Pinus* and *Eucalyptus* to determine whether there is genetic variation for the economic yield response to elevated CO_2 concentrations and identify traits that can be linked to a strong positive response. This should allow the establishment of a detailed program of conventional breeding and marker-assisted selection and also genetic engineering, as is currently underway in food crop science [29], to optimize plant productivity and the complex adaptation to stress (Figure 1) [30].

The increase in productivity of forest plantations as a result of increases in CO_2 concentration [31] opens new perspectives in the management strategies of SRF plantations because it might exacerbate nutrient deficiencies over multiple rotation cycles, possibly leading to re-evaluation of number and length of the rotation cycles. Thus, a priority of future research is to assess whether and how productivity might change over time owing to elevated CO_2 concentration and how management of these plantations can be optimized to take into account these variations. However, similar to semi-natural and natural forest, any management strategy for tree plantations needs to consider the entire ecosystem. This is particularly important if tree plantations represent a change in land use and a high potential to result in loss of soil C. Another issue which needs to be investigated is the effect of elevated CO_2 concentration on the commercial quality of the end products. For instance, there is little information about the energy content of biomass produced by forest plantations under elevated CO_2 concentration.

Replication, modeling and upscaling

Even though FACE technology has allowed for considerable expansion of our observational ability, such spatio-temporal dimensions only cover a fraction of forest ecosystem functionality. Thus, one of the main issues in experiments investigating the effect of climate change on plants is how the information collected can be used for predictions on a larger scale. This aspect is particularly relevant to trees because the knowledge of the response of forest ecosystems to future environmental conditions is of primary concern for life on the planet [32]. Contrary to experiments on grasses and crops where the size of the CO_2-enriched area can be reduced to increase the number of experimental plots, we recommend that experiments on forests will be at least 30 m in diameter to contain a representative proportion of the ecosystem and take into account heterogeneities in the canopy and soil. We also recommend that the number of necessary replicates be determined through rigorous statistical analysis, and not driven solely by convenience, logistics or cost. Plot-to-plot heterogeneity (particularly soil properties) must be evaluated to establish the number of plot replicates. For example, hierarchical Bayesian models have the capacity for utilizing heterogeneous data sets from imperfectly replicated studies with both known and unknown sources of variability, permitting integration of information from different scales [33]. The design of the sites and experiments and their outputs should be closely integrated with modeling approaches, including systems biology and ecosystem and climate change modeling. This will maximize the predictive power of the data and further its integration with the formulation and testing of models. To increase the comparability between experiments and modeling, we also recommend that future experiments employ regression-based experimental designs rather than analysis of variance-based designs.

Another aspect needs to be considered: CO_2 experiments that have been established in forest ecosystems so far have imposed a significant increase in CO_2 concentration which is unlikely to reflect the natural increase of CO_2 concentration over a specific period of time. We propose studies using CO_2 gradients in the open field, establishing a minimum number of six to eight plots each having a different CO_2 concentration. This approach simulates growth conditions over a continuous CO_2 gradient between the plots rather than discrete levels and thus should be methodologically refined in favor of gaining mechanistic understanding of nonlinearity in system CO_2 response.

Location and technological aspects

Most of the FACE experiments have been performed in predominant biomes in the United States of America and Europe because of funding and logistics but not in important biomes, such as tropical and boreal forests, that cover large parts of the globe. In particular, our knowledge on the response of tropical forests to elevated CO_2 and climate change is minimal [34], whereas for boreal forests we have gained some limited information from open top chamber experiments only [35]. These biomes should, therefore, be considered high priority locations for future experiments. Formulating a transcontinental agreement possibly funded by an international institution could, in the end, be a mechanism to establish a suite of large-scale experiments in understudied but critical ecosystems such as the tropical and the boreal forests mentioned above.

It is known that one of the limits to establish large facilities in such ecosystems is the cost of CO_2 to carry out the fumigation, in particular for its transportation from the source to the experimental site. To drastically reduce the experimental costs, predominately related to the costs of providing CO_2 itself, we recommend establishing future experimental sites close to an available CO_2 source such as a fertilizer plant (Figure 2). Geological CO_2 resources that exist at several locations around the world and that are utilized commercially for CO_2 are easily accessible. Official agreements with a major European mining company to access large quantities of pure gaseous CO_2 at no cost already exist in Caprese Michelangelo in central Italy, where a single well is capable of producing more than 900 tons of CO_2/h [36]. Waste CO_2 from industrial or bio-industrial processes such as alcohol distillation could be more widely utilized. Such facilities have already been used in a crop FACE in the USA [37]. Using sources such as sugar cane biorefineries close to tropical forest sites is also

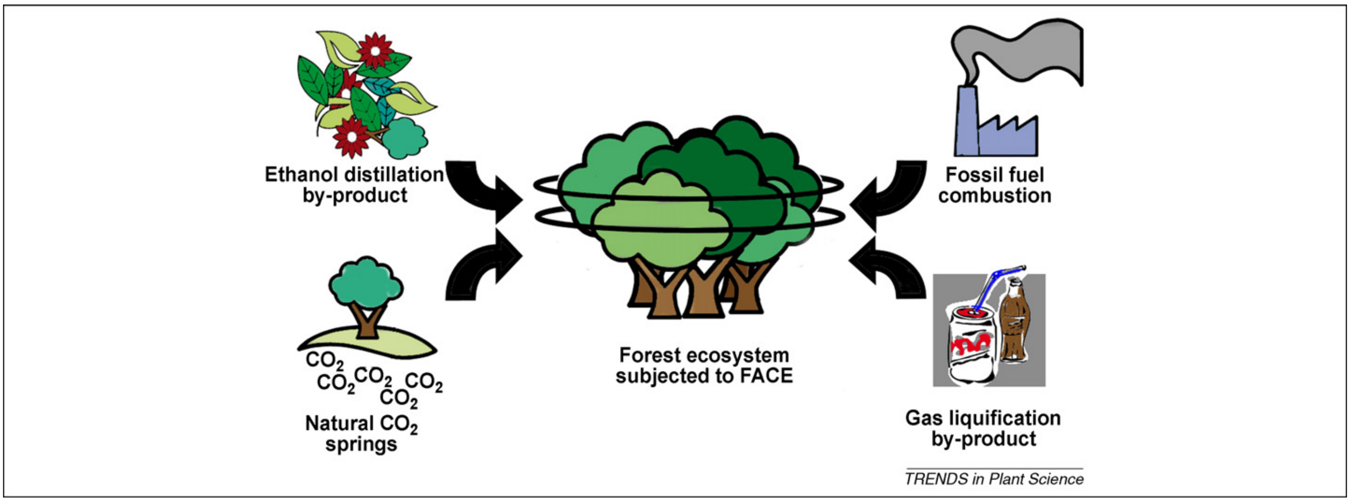

Figure 2. Natural versus artificial CO_2 sources to be considered for future experiments on forests to contain CO_2 costs.

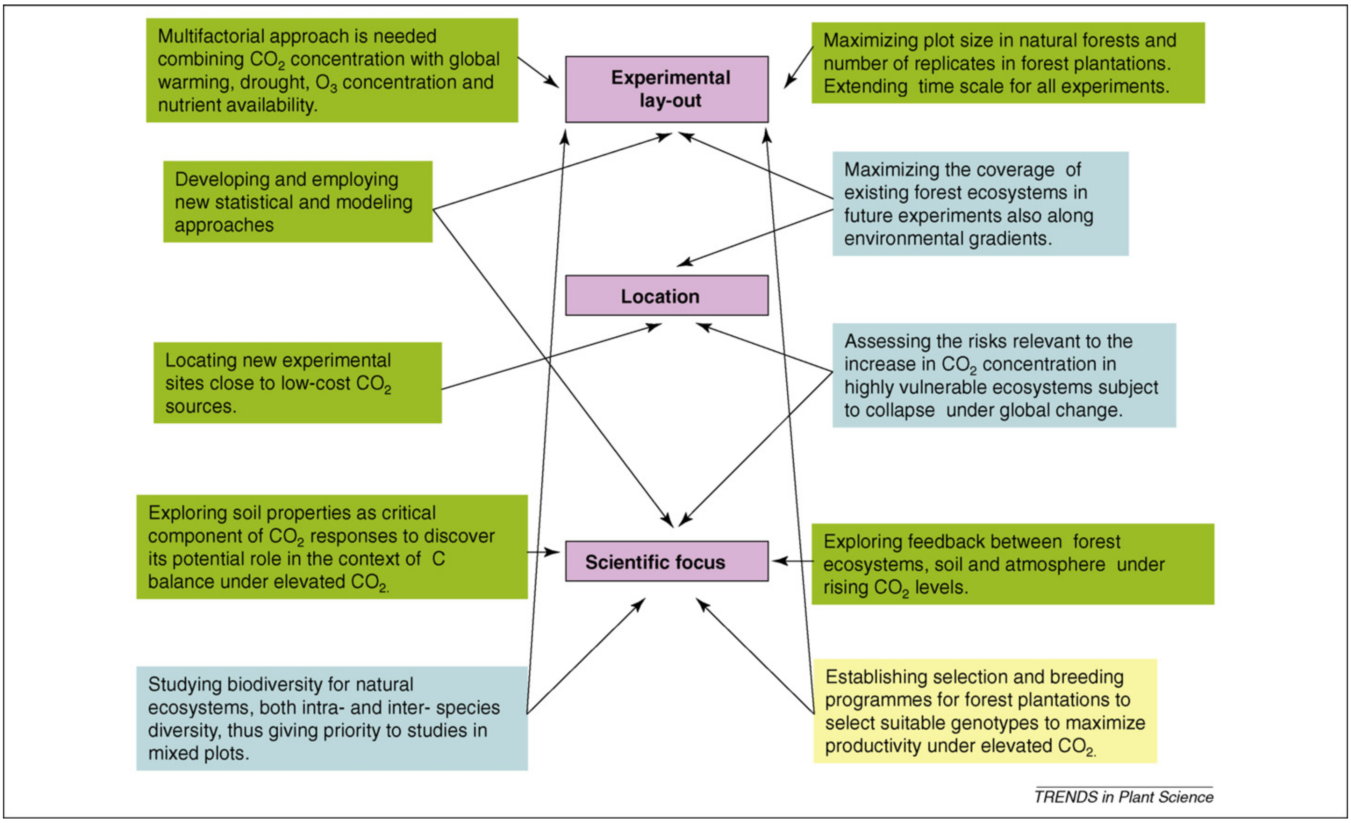

Figure 3. Main priorities and challenges to be considered for the next generation of experiments with forest ecosystems under elevated CO_2 conditions at three different levels: experimental layout, location and scientific focus. Boxes in green refer to both natural forests and forest plantations, boxes in blue refer specifically to natural forests and boxes in yellow refer to forest plantations.

possible. In addition, CO_2 emitted from industries or power stations is a commodity that might be used for large-scale experimentation. Quality might pose a problem on the one hand because of the presence of pollutants or trace gases, but on the other hand because a constant stable isotopic signal ($\delta^{13}C$) value, which is useful for future research programs tracking carbon through the environment, is only present in high-quality samples. However, it seems unlikely that large-scale CO_2 experiments will be realized in the future if a drastic reduction of CO_2 costs will not be achieved.

We also recommend that FACE facilities should be located in association with other studies. Examples include colocation with eddy flux tower facilities or location in areas where biological diversity is actively being manipulated. For many countries which balance between food and non-food production, the importance of modern biomass related research cannot be underestimated.

Concluding remarks and recommendations

The scientific community and policy makers have recognized that the impact of elevated CO_2 concentrations on forest ecosystems deserves particular attention in relation to the other driving forces of climate change. Large-scale and long-term experiments are necessary to establish a comprehensive approach that takes into account the entire ecosystem with all interactions and feedback mechanisms.

FACE studies should proceed beyond descriptive science and recognize the shift to a mandate to understand and predict the consequences of atmospheric and climatic change on the process and ecosystem level to contribute to the design of appropriate strategies to respond to this change.

Furthermore, in Figure 3 we summarize a list of priorities and recommendations that need to be considered by both scientists and funding organizations for future research on forest ecosystems in the context of climate change.

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