Commentary

Is diversity of ectomycorrhizal fungi important for ecosystem function?

One of the most challenging questions faced by ecologists is whether there is a general relationship between biological diversity and ecosystem function. As rates of species extinction appear to be increasing – and evidence has emerged of decreasing biodiversity in plant and microbial communities as a result of air and soil pollution, intensification of agriculture and forestry (Pimm *et al.*, 1995) – the importance of establishing whether biodiversity *per se* is important for ecosystem function has become a central issue in ecology. While the effects of higher plant species diversity on aboveground productivity has received considerable attention (Loreau & Hector, 2001), and there is evidence that arbuscular mycorrhizal (AM) diversity increases plant species diversity and productivity (van der Heijden *et al.*, 1998; Klironomos *et al.*, 2000) the role of ectomycorrhizal (ECM) diversity in ecosystem function, with one exception (Jonsson *et al.*, 2001), has not been experimentally investigated. In this issue, Baxter & Dighton (2001) (pp. 139–149) report on a study designed specifically to answer the question 'does ectomycorrhiza diversity affect plant growth and nutrient acquisition?'.

'Establishing biodiversity–function relationships remains one of the most intractable challenges in ecological research'

The ectomycorrhizal perspective

Increasing awareness of the wide range of mycelial structures produced by ECM in soil and on roots (Agerer, 1996) together with evidence of considerable interspecific and intraspecific variation in production of nutrient-mobilizing enzyme systems by these fungi (Leake & Read, 1997) provides strong theoretical support for ECM diversity increasing effectiveness of nutrient acquisition from different spatial locations and different substrates in the soil. Indeed, it is arguable that any case for biodiversity affecting ecosystem functioning is much stronger for ECM than for AM fungi because of the greater variation in structure and functioning of ECM. Furthermore, as attempts are now being made to derive generalized relationships between plant biodiversity and ecosystem function, using data derived exclusively from studies of herbaceous plant communities that have arbuscular mycorrhizas (Loreau & Hector, 2001) it is increasingly important that the effects of ECM biodiversity on forest ecosystems are investigated.

Ectomycorrhizal diversity effects on plant responses to mycorrhizal associations

Baxter & Dighton (2001) grew *Betula populifolia* seedlings for 10 wk in Petri dishes containing sterilized peat-vermiculite in which the plants were inoculated with 0–4 different species of ECM fungi drawn from a pool of six species. To establish mixed-species communities of ECM fungi in symbiotic association with a plant under axenic conditions is a significant achievement in itself, especially as the species chosen in this case are representative of some of the major different functional and taxonomic groups of the fungi.

The effects of realized ECM diversity on root and shoot biomass, nitrogen and phosphorus contents of the plants were examined. The authors conclude that ECM diversity rather than species composition or rates of mycorrhizal colonization is the main factor affecting responses of the plants (changes in shoot/root biomass allocation and uptake of phosphorus) to mycorrhizal associations. The finding that ECM diversity was positively correlated with the total P uptake by the plants lends support to the hypothesis advanced by Roger Koide, in relation to AM fungi, that diverse mixtures of mycorrhizal fungi may provide functional complementarity in the uptake of P, enabling more effective and complete exploitation of soil P (Koide, 2000; Smith *et al.*, 2000).

While the most diverse communities of ECM assembled by the authors contained only four species, which is low compared to the typical diversity seen in established forests (Dahlberg, 2001), this level of diversity is appropriate for studies of tree seedlings. However, the pool of species drawn upon in this case (six species) is very low. Kranabetter & Wylie (1998), for example, found that 4-yr-old naturally regenerating seedlings of *Tsuga heterophylla* growing in forest gaps had a mean of 3.8 ECM species-morphotypes per seedling in the centre of the gaps rising to 6.1 species per

seedling under the undisturbed forest canopy. However, in this natural situation the pool of species with which the seedlings were infected was much greater, ranging from 20 species in the clearings to 32 under the closed forest.

Although Baxter and Dighton (2001) is a pioneering paper, it is premature to draw general conclusions about links between diversity and functioning of ectomycorrhizas from it. The authors acknowledge the implications of the study are constrained in part by the artificiality of the experimental system, but in addition, as in many previous studies of biodiversity–ecosystem function relationships, there are potentially confounding factors that make the interpretation of the results and their wider significance problematic. Nonetheless, this is a timely paper that has the potential to stimulate further work in this very difficult field of research and it usefully highlights some of the experimental problems that need to be overcome.

The problem of confounding factors

In their study, Baxter & Dighton (2001) were careful to design an experiment to avoid two of the pit-falls encountered in previous biodiversity–function studies (Huston, 1997): they randomly allocated species to treatments and they tested the effects of each ECM species in symbiosis with the plant to enable the effects of species richness to be distinguished from those of species composition. Unfortunately, probably as a result of the experimental conditions in which the plants were grown in a small volume of peat and vermiculite to which relatively high concentrations of mineral nutrients were added at the start of the experiment, the effects of ECM on growth and nutrition of the plants were, with few exceptions, not significant. Consequently, virtually no species-specific effects can be discerned in this case.

The final communities of ECM that were assembled were not strictly random, despite the care taken in the experimental design, since two species were responsible for most of the cases in which infections were not established. As the number of ECM fungi inoculated onto the *Betula* seedlings was increased from one to two and then to four there was a progressive decrease in effectiveness of establishment of all inoculated species from 100% to 86% and 52%, respectively. Thus as community diversity increases there appears to be increasing bias in the species combinations that successfully infect the plants simultaneously. This problem is hard to avoid. It may be possible to employ methods of inoculation that reduce the rates of failure to form mycorrhiza, but the decline in numbers of root tips infected by each species as diversity is increased is predictable, and clearly shown in this paper and in Jonsson *et al.* (2001). Assembly of 'random' communities of ECM fungi becomes increasingly difficult at higher levels of biodiversity and this confounds attempts to distinguish biodiversity and species composition effects.

A second confounding factor that can be easily remedied in future studies is the method of inoculation, which differed across the diversity treatments. In the single species replicates, ECM inoculum was placed as a single patch in the centre of each Petri dish in which the tree roots were grown. In the two-species combinations, two spatially separate patches of inoculum were added and in the four-species combinations four spatially separate patches of inoculum were added. The dispersal of the inoculum therefore progressively increased in parallel with the increased diversity. The authors report increases in mycorrhizal colonization of the seedlings with increasing diversity of ECM. Although the strength of correlations between species diversity and plant responses were stronger than the effects of ECM colonization, we cannot exclude the possibility that these effects are due to the different methods of inoculation.

Future directions

The recent advances in knowledge of mycorrhizal community structures achieved through morphotype and molecular identification of ECM on roots (Dahlberg, 2001) provides a much firmer basis on which to select species for inclusion in future studies of biodiversity and function. The size of the species pool and the numbers of species combinations used (the level of diversity) can increasingly be based on knowledge of natural ECM communities. As the abundance of species that rarely or never produce visible fruiting structures has become apparent (Dahlberg, 2001) increased efforts will need to be made to include them in biodiversity and functional studies.

Clearly too, it is important that experimental conditions are employed that provide the kinds of spatial heterogeneity and variation in forms of nutrients that occur in the field, since this is likely both to result in significant benefits to the plants from their mycorrhizal associations and to reveal any 'complementarity' of ECM species in accessing different nutrient pools. One possible way to achieve this may be to establish communities of ECM on tree seedlings in soil-based microcosms in which natural sources of organic and inorganic nutrients are supplied (Timonen & Sen, 1998).

Establishing biodiversity-function relationships remains one of the most intractable challenges in ecological research. Ectomycorrhizal communities tend to be increasingly diverse as tree seedlings grow, become established and finally become part of mature forests (Kranabetter & Wylie, 1998). It is therefore likely that any general relationship between ECM diversity and ecosystem function will be most important in mature forests, but artificial reconstruction of these communities with different levels of ECM diversity does not appear to be a realistic experimental approach. Seedlings are clearly more amenable than trees to such studies, but there is increasing evidence that the range of ECM diversity that can be supported by individual seedlings is quite small

(Kranabetter & Wylie, 1998), almost certainly as a result of the constraint provided by the limited carbon-fixing capacity of seedlings compared with established trees.

Concluding remarks

Investigations of the cost-benefit relationships of ectomycorrhizal diversity are a high priority. How are the competing demands for carbon by different ECM met by the plants? Is more carbon allocated proportionally to the partners that provide the most nutrients or other functional benefits? Does ECM diversity have any consistent effect either on individual plants or on ecosystem functioning? These questions are easy to pose. The answers are difficult to obtain. A major challenge lies ahead.

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Letters

Transient ecosystem responses to free-air CO_2 enrichment (FACE): experimental evidence and methods of analysis

How is it possible to extrapolate results from field CO_2 experiments to allow the prediction of large-scale, long-term responses of ecosystems to global change? There are two key points of view on this long-standing issue. The first considers that currently running experiments using free-air CO_2 enrichment (FACE) facilities or open-top chambers mimic a future atmospheric CO_2 concentration (C_a), so that observations from the experiments represent ecosystem responses to the designated CO_2 level. However, here both theoretical (Luo & Reynolds, 1999) arguments and experimental (from the Duke Forest FACE experiment) evidence are put forward to support a second viewpoint. In this, the current experiments are considered to exert an ecosystem perturbation, achieved primarily by altering carbon (C) influx. Observation of ecosystem responses to the perturbation helps probe the processes

and mechanisms. If this second viewpoint is correct, then we must also ask how we can use results from FACE experiments, where ecological responses to a rather abrupt perturbation are measured, in order ultimately to understand and predict long-term ecosystem responses to a very gradual CO_2 change in the real world.

Transient responses to free-air CO_2 enrichment

FACE facilities have become a premier approach for conducting ecosystem CO_2 experiments (Hendrey *et al.*, 1999; Miglietta *et al.*, 2001; Okada *et al.*, 2001). They have been implemented in numerous ecosystems, including an agricultural field in Arizona (Kimball *et al.*, 1994), a loblolly pine forest at Duke University in North Carolina (DeLucia *et al.*, 1999), a pasture in Switzerland (Van Kessel *et al.*, 2000), the Mojave Desert in Nevada (Jordan *et al.*, 1999), a sweetgum forest in Tennessee (Norby *et al.*, 2001), a grassland in Minnesota (Reich *et al.*, 2001), a poplar plantation in Italy (Miglietta *et al.*, 2001), and a rice field in Japan (Okada *et al.*, 2001). There is tremendous enthusiasm for FACE technology among ecologists around the world because it provides an unprecedented opportunity to conduct manipulative CO_2 experiments in intact ecosystems with minimal environmental disturbance. The FACE experiments have already yielded a great deal of useful information on ecosystem function and are one of the most effective ways to gain short-term insights into long-term issues.

The FACE experiments are inspired primarily by the phenomenon of rising C_a in the real world. Ultimately, we have to extrapolate experimental results beyond the FACE plots to infer the capacity of terrestrial ecosystems to sequester C. The extrapolation is, however, complicated by the fact that the FACE experiments impose a step increase in CO_2 concentration whereas ecosystems in the real world are experiencing a gradual increase in C_a. Therefore, the first issue we need to address regarding extrapolation is how comparable the ecosystem responses to a gradually increasing C_a are to the responses to the step CO_2 increase. Luo & Reynolds (1999) conducted a modelling study to address that issue. Their modelling study indicates that when CO_2 increases gradually as in the real world, the ecosystem C sequestration rate increases gradually. In response to a step CO_2 increase, ecosystem C sequestration rate is predicted to increase abruptly by approximately 10-fold and then decline gradually. Similarly, the step and gradual CO_2 increases give contrasting patterns of N cycling. The predicted transient responses to a step CO_2 increase are consistent with other modelling results (Comins & McMurtrie, 1993; Rastetter *et al.*, 1997; Cannell & Thornley, 1998).

In addition to physiological transition (e.g. bud primordia set under ambient CO_2) to elevated CO_2 (Norby *et al.*, 2001), the transient responses to the step CO_2 increase are primarily structural consequences of ecosystem C and N processes. Ecosystem C sequestration rate at a given time is the difference between ecosystem C influx via photosynthesis and efflux via respiration. In response to a step CO_2 increase, photosynthesis immediately increases due to its fast response time. Respiration is generally proportional to C pool sizes in plants, litter, and soil organic matter (SOM). In response to the step CO_2 increase, changes in pool sizes are cumulative, resulting in a gradual increase in ecosystem respiration. The difference between the step increase in photosynthesis and the gradual increase in respiration translates to a transient response in C sequestration rate, which abruptly increases immediately after the CO_2 fumigation and then gradually declines. In response to a gradual C_a increase, both photosynthesis and respiration gradually increase with a time lag in respiration. The time lag creates a difference between photosynthesis and respiration at any given time, resulting in a gradual increase in the ecosystem C sequestration rate over time (Luo & Reynolds, 1999).

Experimental evidence

While it is not feasible to conduct an experiment lasting hundreds of years in the real world to observe ecosystem responses to a gradual C_a increase, we can use experimental data to verify the processes causing the transient responses. The processes are the step increase in photosynthetic C influx and the gradual increase in respiratory C release in response to a step CO_2 increase as in FACE experiments. Here, I present experimental evidence from the Duke Forest FACE experiment to demonstrate an immediate, step increase in ecosystem C influx accompanied by a gradual increase in ecosystem C efflux via respiration.

The Duke FACE experiment is an on-going project – started in August 1996 – and has been well described in terms of experimental design and facility (Hendrey *et al.*, 1999), climate (Luo *et al.*, 2001a), and soil properties (Andrews & Schlesinger, 2001). Leaf-level photosynthesis at the Duke Forest FACE increased by approx. 40–60% in the past 5 yr (Ellsworth, 2000). In order to provide a whole-ecosystem estimate of C influx, we used the MAESTRA model to estimate photosynthetic CO_2 assimilation of the loblolly pine canopy at the FACE site (Luo *et al.*, 2001a). MAESTRA is a three-dimensional model of forest canopy radiation absorption, photosynthesis, and transpiration. It incorporates standard formulations of the mechanistic C_3 photosynthesis model of Farquhar *et al.* (1980) and a more empirical formulation of stomatal conductance described by Ball *et al.* (1987). The canopy is represented by an array of semiellipsoidal tree crowns. Each crown is divided into six horizontal layers with each layer divided into 12 gridpoints of equal volume. Each layer is specified by a number of physical and physiological properties, including radiation, temperature, leaf area, and leaf N content. Environmental variables that drive model simulations are radiation, air temperature, air humidity, wind speed and C_a above the canopy.

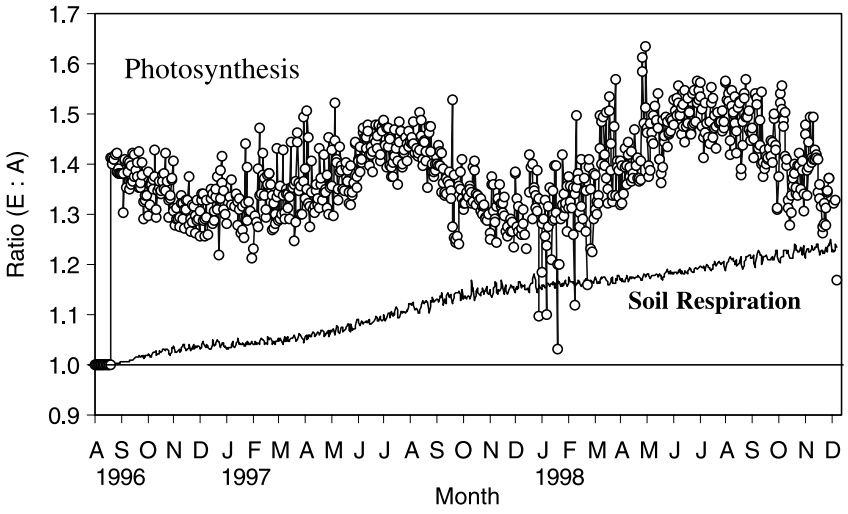

Fig. 1 Results from the Duke Forest FACE experiment demonstrate contrasting patterns of ecosystem photosynthetic (open circles connected with line) vs respiratory (line) responses to the step CO_2 increase. The ratio of modelled daily canopy C fluxes at elevated relative to that at ambient CO_2 from August 1996 to December 1998 displays a step CO_2 stimulation of C influx (Luo *et al.*, 2001a). However, the step increase in the C influx did not lead to a step increase in respiratory C release. Rather the ratio of modelled soil respiration shows little change in the first year but a gradual increase in the second and third years under the elevated CO_2 treatment compared to that under the ambient CO_2 treatment (Luo *et al.*, 2001b).

The MAESTRA model was validated against measurements of leaf-level photosynthetic rates and canopy photosynthesis derived from eddy-covariance measurements at the FACE site (Luo *et al.*, 2001a). The validated model predicts that daily canopy C uptake ranged from 0 g C m^{-2} d^{-1} in the winter on exceptionally cold days to 8 g C m^{-2} d^{-1} in the summer in the ambient CO_2 plots. In elevated CO_2 plots it ranged from 0 g C m^{-2} d^{-1} in the winter to nearly 12 g C m^{-2} d^{-1} in the summer. Accordingly, elevated [CO_2] increased average ecosystem C influx by approx. 40% following the CO_2 fumigation in August 1996. The CO_2 enhancement in canopy C influx displays a step function, indicating an abrupt, large increase in C influx into the forest ecosystem immediately after the CO_2 fumigation (Fig. 1).

The step increase in C influx into the elevated CO_2 plots did not lead to a step increase in C efflux via respiration. Soil respiration rates (including both root and soil microbial respiration) were measured approximately monthly at the Duke FACE site (Andrews & Schlesinger, 2001). Measured midday values of soil respiration at the site displayed a strong seasonal variation, 0.05 g C m^{-2} h^{-1} in winter and 0.4 g C m^{-2} h^{-1} in summer. Elevation of CO_2 concentration did not result in a statistically significant difference in the soil respiration in 1996 but led to significant increases of 23.2% and 35.5% in 1997 and 1998. The observed gradual increase in soil respiration is qualitatively consistent with the model predictions (Luo & Reynolds, 1999).

In order to improve the mechanistic representation of soil C processes as affected by environmental variables, Luo *et al.* (2001b) modified the Terrestrial C Sequestration (TCS) model, which simulates soil respiration. The model uses the ecosystem C influx as an input, which is partitioned into leaf, wood, and fine roots. Litter from those biomass pools is decomposed by microbes, resulting partly in release of CO_2 and partly in formation of SOM. SOM is mineralized to release CO_2. Since various rhizosphere C processes possess different response times (or residence time, the mean from C entry via photosynthetic C fixation to exit via respiratory C release), photosynthetically fixed C is released back to the atmosphere via respiration with a range of time lags behind photosynthesis itself. The respiratory C release from the soil surface is the convolution of root respiration and microbial decomposition of litter and SOM from various pools with different time constants (Luo *et al.*, 2001b). Thus, the step increase in photosynthetic C influx is transformed into a gradual increase in respiratory C release.

Preliminary results also suggest a gradual increase in plant respiration under elevated CO_2 at the Duke FACE site. Measured specific respiration rates in trunks and needles showed little CO_2 effect (J. Hamilton & E. DeLucia, pers. comm.) whereas measured plant biomass in elevated CO_2 showed a gradual increase since the onset of the FACE experiment compared with that in ambient CO_2 (DeLucia *et al.*, 1999), resulting in a gradual increase in plant respiratory C release.

Integration of the three components of C processes (i.e. canopy photosynthesis, plant and soil respiration) yields a large C sequestration in the first year of the FACE experiment, followed by smaller net C storage in the second and third years (Fig. 1). This dynamic pattern of C sequestration is qualitatively consistent with that predicted by Luo & Reynolds (1999).

Methods of analysing transient responses

Both model simulations and experimental data support the argument that the FACE experiments generate a perturbation to ecosystems, primarily by altering input of C flux. This type of experiment requires that a decent size of perturbation be imposed on ecosystems in order to trace responses in a reasonable time frame. As in most system research, we must analyse results from the perturbation experiments in such a way that we can characterize ecosystem structure and estimate parameter values before prediction of future changes.

The structure of ecosystem C processes can conceptually be characterized by compartmentalization, donor-controlled transfer, and sequential linearity (Luo & Reynolds, 1999). The C processes are highly compartmentalized due to the fact that photosynthetically fixed C goes to distinctive compartments, such as plant, litter, and SOM. Donor-controlled transfer is reflected by the fact that C release from each compartment through plant and microbial respiration is controlled by sizes of donor pools and hardly by products of respiration. In addition, the majority of photosynthetically fixed C sequentially transfers from one compartment to another, following a first-order linear function (Bolker *et al.*, 1998). Only a small fraction of C can be recycled between SOM and soil microbes. The three properties have been incorporated in virtually all biogeochemical models (Jenkinson & Rayner, 1977; Parton *et al.*, 1987; Comins & McMurtrie, 1993; Rastetter *et al.*, 1997; Thompson & Randerson, 1999). Although the three properties are derived from experimental evidence, the model structure of C processes is yet to be rigorously tested (Luo *et al.*, 2001b).

The parameters we need to know in order to predict ecosystem responses to gradually rising C_a in the real world include ecosystem C influx, C partitioning coefficients among pools, and transfer coefficients from donor pools. It is technically difficult to measure ecosystem-scale C influx, particularly in the elevated CO_2 plots even though leaf photosynthesis can be easily measured. An alternative method for estimating ecosystem C influx is modelling synthesis of experimental data, which also allows us to quantify the stimulation of canopy C uptake under elevated CO_2. While extensive leaf-level measurements have been made in almost all FACE experiments and a variety of canopy models are available, synthesis of data from FACE experiments with canopy models is not only technically feasible but also has the potential to make a critical contribution to predicting C sequestration. The challenge is how to organize resources to realize the potential.

Parameter estimation for C partitioning and transfer coefficients becomes more challenging than that for ecosystem C influx due to lack of experimental data and methodological difficulties. According to the degree of difficulty, derivation of parameter values from data can be divided into four cases. First, experimental data can be directly converted to parameter values. For example, specific rates of litter decomposition can be derived directly from laboratory and field studies on litter decomposition. Second, measured values represent results of two or more simultaneous but counteracting processes. For example, fine root biomass at a given time is determined by the balance between root growth and death, which occur simultaneously but counteractively. Parameter estimation for such processes depends on ancillary information. Third, parameter values are not measurable in experiments due to limited technology. For example, root exudation, which is suspected to be an important pathway of C flow to the rhizosphere (Hu *et al.*, 1999), is not readily measurable in natural ecosystems. Parameterization is largely based on an educated guess. Fourth, a measurable quantity is a convolution of several processes with distinguishable characteristics. For example, soil respiration is regulated by multiple processes, including root exudation, root respiration, root turnover, and decomposition of litter and SOM. Those processes have distinctive response times to C perturbation. For this kind of data, deconvolution is an effective approach to parameter estimation (Luo *et al.*, 2001b).

A quantitatively rigorous approach to estimating parameter values is inverse analysis, which has proved to be a very powerful tool for model-data integration in other scientific disciplines. The inverse analysis is an approach that fundamentally focuses on data analysis for tests of model structure and parameter estimation. It is often used interactively with forward analysis, which is usually implemented using simulation models. The latter predicts ecosystem responses to global change with a given model structure and a set of prescribed parameter values. Generally speaking, the forward analysis asks what a model can tell us about the ecosystems whereas the inverse analysis asks what the data can tell us about the same system. Combination of the two approaches allows us to probe mechanisms underlying ecosystem responses to elevated CO_2. Since simulation modelling is familiar to most researchers, here I will outline the general procedure of the inverse modelling and relevant data requirements.

Inverse modelling usually starts with collection of experimental data (step 1) with an attempt to ask what the observed responses to a perturbation can tell us about the system in question (Fig. 2). By combining prior knowledge

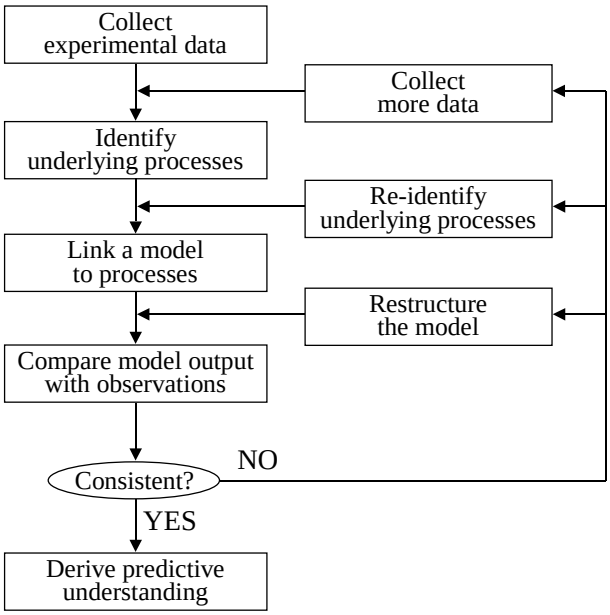

Fig. 2 General procedure of inverse analysis to test model structure and derive parameter values from experimental data.

about the system, we try to identify processes underlying the observations (step 2). With major processes identified, we can develop a model to link these processes according to ecological mechanisms (step 3). The model can be used in the forward analysis to predict ecosystem responses to global change and can be used in the inverse analysis as well (step 4). The latter is usually implemented with mathematical algorithms for comparison of model predictions with the observed responses (White & Luo, 2001). By comparing model predictions with observations, step 4 attempts to challenge model structures against and/or to derive parameter values from data so that predictions of the system's behaviour are improved. Steps 1–4 are generally iterated several times until we find a model structure that adequately represents the system and then estimate parameter values that quantify interactions and feedbacks. The inverse analysis eventually results in improvement of our ability to predict future changes of the system (step 5). Although the inverse analysis has been hardly discussed in the literature of ecology, it was applied to photoacoustic signals (Tabrizi *et al.*, 1998), population dynamics (Wood, 1997), seed dispersal (Clark *et al.*, 1999), and rhizosphere C processes (Luo *et al.*, 2001b).

Inverse analysis requires accurate data, which may derive from proper experimental design and effective measurement plans. Most FACE experiments set two CO_2 levels, for example one at ambient CO_2 and the other at ambient +200 ppm, a C_a level 40–60 yr from now. In such a perturbation experiment, generation of a perturbation size that is effective to probe mechanisms shall be one of the primary goals of experimental design. The perturbation sizes in a FACE experiment can be measured as the amount of additional C influx into an ecosystem at elevated CO_2 in comparison to that at ambient CO_2. The amount of additional C influx under elevated CO_2 is related to productivity of an ecosystem and the CO_2 treatment level. In order to obtain most useful experimental results from FACE experiments, it may even become desirable to estimate the perturbation size before we implement a FACE experiment.

In the past, a substantial effort has been made on measurements of leaf physiology. Without integration of leaf-level data into estimation of canopy C influx, measurements are not useful for predicting ecosystem C sequestration. Probably because of difficulties in methodology, data on soil C and N processes are less available. Characterization of ecosystem C processes requires parameter estimation of partitioning coefficients among C pools, pool sizes, and C transfer rates from the pools. In order to quantify those parameters, it is imperative to observe time courses of fluxes and pool sizes following the perturbation of step CO_2 changes.

Uncertainties in experimental data make parameter estimation difficult. Quantification of C fluxes through different pathways by inverse analysis relies heavily on accuracy of data. All the FACE experiments are implemented in field with natural variability in environmental and biological factors. The field experiments have inherent complications, such as successional changes in ecosystem processes and episodic events of precipitation in deserts. In addition, FACE experiments starting with saplings (Miglietta *et al.*, 2001) carry in developmental variation. CO_2 experiments in agricultural fields may be accompanied with other disturbances, such as fertilization, fallow, and ploughing. Those factors all potentially obscure actual effects of elevated CO_2 and cause tremendous difficulties in data analysis. How to reduce background variability and increase accuracy of data should be one of the major considerations in designing future FACE experiments.

Summary

Ecosystem responses to the perturbation generated by a step CO_2 increase in field CO_2 experiments are different from those to a gradual C_a increase as in the real world. In order to develop our ability to predict ecosystem responses to a gradual C_a increase, we need to analyse data from FACE experiments using an inversion approach to challenge the structure of existing models and derive parameter values. In order to effectively conduct the inverse analysis, we need to collect highly accurate, informative data by improving experimental design and measurement plans for the FACE studies.

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