Commentary

Myco-heterotroph–fungus marriages – is fidelity over-rated?

'Full appreciation of the evolution of myco-heterotrophy will remain elusive until the key evolutionary parameter – fitness – is measured'

Though green coloration is a defining feature of the plant kingdom, there are many nongreen (i.e. achlorophyllous/nonphotosynthetic) plants which have long sparked the curiosity of botanists (see Fig. 1). These plants can be divided into two functional groups: those that directly invade other plants to acquire food, such as the mistletoes, and those that do not. Members of the latter group have historically been called 'saprophytes' but are more properly labeled 'myco-heterotrophs', a term which highlights the fact that they acquire all their fixed carbon from mycorrhizal fungi (Leake, 1994; see also Fig. 1). Let's be clear – we are talking about plants that consume fungi. As odd as such a lifestyle may sound, at least 400 myco-heterotrophic species are distributed across nine families of monocots and dicots (Furman & Trappe, 1971; Leake, 1994). The 'crown jewels' of the myco-heterotrophs are the orchids, of which the estimated 30 000 enchanting species encompass nearly 10% of the Angiosperm flora. Of course, the vast majority of orchids are photosynthetic, at least as adults. However, all orchids can be classified as partially myco-heterotrophic because their minute 'dust seeds' lack energetic reserves and must locate a fungus on which to feed during the interval between seed germination and the elaboration of photosynthetic organs months or years later. In addition, multiple independent lineages of terrestrial orchids have given up photosynthesis entirely, becoming 'fully myco-heterotrophic.' Members of another widely distributed and well known myco-heterotrophic subfamily, the Monotropoideae (Ericaceae), share many convergent attributes with orchids (Leake *et al.*, 1994). Myco-heterotrophs interact in a physiologically intimate fashion with specific fungal partners, providing amusing opportunities for analogies with human relations (Gardes, 2002). Papers in this issue by McCormick *et al.* (pp. 425–438) and Leake *et al.* (pp. 405–423) provide important new insights into the 'marriages' between myco-heterotrophs and their fungal partners. In particular, these papers demonstrate high fidelity of the plants across all life stages, with the glaring exception of one orchid species which switches partners.

The infidelity problem

Ordinary mycorrhizal interactions involve a reciprocal exchange of photosynthetically fixed plant carbon in return for fungally scavenged soil minerals, and are thus regarded as mutualisms. Most plants display no signs of fidelity to particular fungal partners. For example, ectomycorrhizal Douglas fir has been estimated to associate with at least 2000 fungal species which span tens of families of Ascomycetes and Basidiomycetes (Molina *et al.*, 1992). By contrast, idiosyncratic and specific fungal associations were described in orchids by the turn of the 20th century (Bernard, 1909), and were shortly thereafter suggested in the Monotropoideae as well (Francke, 1934). Other authors disagreed vociferously with these claims of specificity (Curtis, 1937; Hadley, 1970). In the case of orchids, some of the disagreements can be blamed on the predominance of associations with fungi in the problematic 'taxon' *Rhizoctonia*. This form-genus encompasses distantly related clades of fungi which rarely fruit in culture, are difficult to identify based on vegetative characteristics, and can interact with plants as mycorrhizae, endophytes or parasites.

Recent molecular studies have confirmed mycorrhizal specificity in several fully myco-heterotrophic orchids (reviewed in Taylor *et al.*, 2002; see also Selosse *et al.*, 2002b; Taylor *et al.*, 2003; Taylor *et al.*, 2004). A parallel series of studies has clarified the fungal associations of most members of the Monotropoideae and documents equal or greater specificity than that found in orchids (Bidartondo & Bruns, 2001, 2002). This specificity is perplexing, since eschewing potential partners must come at a cost. One potential explanation has been presented as follows (Cullings *et al.*, 1996; Taylor & Bruns, 1997). Fungi form mycorrhizae in order to acquire carbon, and yet myco-heterotrophs remove carbon rather than providing it to their fungal partners. Hence, they can be viewed as parasites upon their

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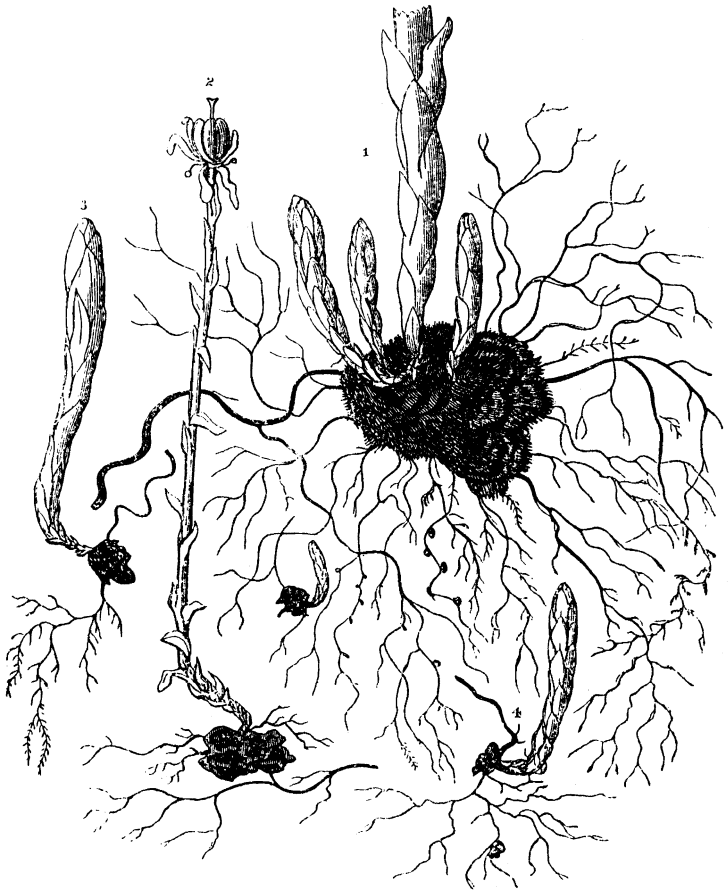
ART. XXXV.— *On the parasitic growth of Monotropa Hypopitys.*
By Edwin Lees, Esq., F.L.S., &c.



ILLUSTRATIONS OF THE MODE OF GROWTH OF MONOTROPA HYPOPITYS.

1. Base of a mature plant, 14 inches high, and three young unexpanded plants, growing from their radical parasitical knob. 2. Smaller plant in seed 3, 4 & 5. Young plants growing from radical knobs.

Fig. 1 The progenitor of this journal, *The Phytologist*, was an important early forum for discussions and observations on the parasitic nature of *Monotropa hypopitys* (Lees, 1841). The plant had been assumed to be a typical angiosperm parasite by Linnaeus, but Luxford (1841), Lees (1841), and Rylands (1842a) were perplexed to find no evidence of haustorial attachments to other plants. Their observations, including recognition of fungal mycelium ensheathing its roots (Rylands, 1842b), paved the way for the studies of myco-heterotrophic germination and development of *M. hypopitys* and identification of its fungal partners now reported in this issue.

fungi. Parasitism tends to favor specificity because of selection on victims to resist their attackers, and ensuing evolutionary 'arms-races'. These arguments lead to the prediction that fully myco-heterotrophic orchids should be more specific than green orchids. McCormick *et al.* put this prediction to the test by carefully documenting specificity in three photosynthetic terrestrial orchids using modern molecular-phylogenetic and seed-packet germination field trials and comparing their results to specificity in a previously studied fully myco-heterotrophic orchid.

Difficulties in finding suitable partners

McCormick *et al.* isolated fungi from individual coils (pelotons) of mycorrhizal fungi teased out of root cells of *Goodyera pubescens* and *Liparis lilifolia*. They then amplified and sequenced several diagnostic ribosomal gene regions, including the highly variable ITS. All of the fungi from these orchids belonged to the genus *Tulasnella*, a member of the *Rhizoctonia* complex commonly found in orchids worldwide. Remarkably, the 10 isolates from *Liparis* displayed a maximum of approx.

0.2% sequence divergence, suggesting that *L. lilifolia* associates with only a single fungal species over a wide geographic area. Isolates from *Goodyera* were slightly more diverse, but still closely related when compared to the ITS sequence diversity of tomentelloid fungi found associated with the fully myco-heterotrophic orchid *Cephalanthera austinae* in a previous study (Taylor & Bruns, 1997). McCormick *et al.* contribute an additional key piece of information. They found that germinating seeds and protocorms of these two species from packets planted in the field associated with the same narrow clade of fungi as did adult plants. Hence, we see lifelong fidelity in these two photosynthetic orchids, which has also been demonstrated recently in several fully myco-heterotrophic orchids (McKendrick *et al.*, 2000; McKendrick *et al.*, 2002). The results of McCormick *et al.* are counter to the predictions about fidelity in photosynthetic vs nonphotosynthetic plants and therefore require a re-examination of orchid-fungus marriages. However, these species may also depend heavily on fungally supplied carbon – they produce single leaves (some in winter) and grow on the dusky floors of dense forests. In this context, Otero *et al.* (2002) have recently reported low ITS sequence diversity among the *Ceratobasidium* associates of several epiphytic orchids. One would not expect significant myco-heterotrophic carbon gain by adult orchids in the forest canopy, though this possibility deserves examination (Ruinen, 1953).

The third species studied by McCormick *et al.*, *Tipularia discolor*, stands in stark contrast to the first two. *Tulasnella* isolates, along with other fungi from *Tipularia* adults, were phylogenetically diverse. Hence, this orchid appears to display relatively low specificity in the adult stage. Does this mean *Tipularia* establishment is unlikely to be constrained by a lack of suitable partners? Not at all. Perhaps the most exciting result reported by McCormick *et al.* is that wild *Tipularia* protocorms associate with a narrow range of fungi that are not *Tulasnella* species, nor any kind of *Rhizoctonia*. These fungi could not be isolated, but were characterized using direct molecular approaches. These fungi are relatively distant from any species that have been sequenced and deposited in the public databases, but appear to be allied to the Auriculariales. This order of Basidiomycete jelly fungi includes many wood decomposers. Coincidentally, germination of *Tipularia* seeds seems to occur predominantly, if not exclusively, in decaying wood. Therefore, establishment of this widespread orchid is likely to require both a specific fungal clade and a specific microhabitat.

Previous studies have shown that adult *Monotropa hypopitys* in North America have complete fidelity to fungi in the genus *Tricholoma* (Bidartondo & Bruns, 2002). *Tricholoma* is ectomycorrhizal, and hyphally links *Monotropa* to its ultimate carbon source – autotrophic hosts such as *Salix*. Leake *et al.* set out to determine whether the natural distribution of the fungal partners and autotrophic hosts influence the germination and growth of *Monotropa* seeds. Many thousands of dust seeds contained in hundreds of mesh packets were introduced into two sites with adult *Monotropa* plants. This method immobilizes the miniscule seeds, permitting their recovery from the soil, while also permitting hyphal entrance and interaction with the seeds. At the first site, packets were planted in two microhabitats: near adult plants and at least 5 m distant from any observed adults. Considerable germination and seedling growth occurred in plots near adults. Though some germination occurred away from adults, no appreciable growth occurred. At the second site, seed packets were again planted in two microhabitats: under *Salix* and in interspersed, open grassy areas. Germination and growth were highly variable under *Salix*, as might be expected if *Tricholoma* is patchily associated with its autotrophic host, and essentially absent in the grassy areas.

In addition to these detailed studies of *Monotropa* seed germination, molecular analyses of the fungal associates of seedlings and adults were conducted. Leake *et al.* found absolute fidelity to *Tricholoma cingulatum* in both seedlings and adults growing with *Salix*, and fidelity to the closely related *Tricholoma terreum* in adults growing under *Pinus*. Interestingly, they note that neither of these *Tricholoma* species is particularly abundant in the *Salix* and *Pinus* ecosystems, according to fruiting records. The results presented by Leake *et al.* provide the strongest evidence to date that the distribution of a single fungus can forcefully constrain the establishment and resulting distribution of an Angiosperm. These findings have obvious and important implications for the conservation and management of threatened myco-heterotrophs. The findings of equally high specificity at the protocorm stage in photosynthetic orchids dramatically widens the conservation implications.

Recommendations for coping with infidelity

In the decade since the seminal *New Phytologist* Tansley Review of myco-heterotroph biology by Leake (1994), many vexing problems have been clarified, particularly relating to fungal identities, linkages to autotrophs and seed germination in the field, in prominent papers including the two in this issue. Yet, a number of the key questions posed by Leake (1994), and more recently by Gardes (2002), remain unresolved.

The utilization of modern molecular phylogenetic approaches to characterise specificity has provided major advances (Cullings *et al.*, 1996; Taylor & Bruns, 1997; Selosse *et al.*, 2002b; Bidartondo *et al.*, 2003). However, as researchers dig more deeply into specificity, increasingly quantitative methods for comparative analysis will be needed. The tools of phylogenetics and population genetics offer a variety of options by which we may summarize genetic diversity within a set of fungal associates using a single statistic (Taylor *et al.*, 2004), which would be preferable to *ad hoc* comparisons based on tree topologies alone. These quantitative values can then be compared across species, populations, geographic regions, and so forth. Specificity toward two or more clades

of fungi could also be summarized using these statistics. The transitions between protocorm and adult stages in *Tipularia* make it clear that future studies must differentiate life cycle components of specificity. Statistical evolutionary methods will also become increasingly important as we begin to reconstruct the history of mycorrhizal associations in major groups such as the Orchidaceae and Ericaceae. A key question which is just appearing on the research horizon concerns the possible role of switches of fungal partners in the diversification of myco-heterotrophic (and other?) plant lineages. Unfortunately, a full appreciation of the evolution of myco-heterotrophy will remain elusive until the key evolutionary parameter – fitness – is measured in both plant and fungus under a variety of conditions. This is perhaps the greatest challenge facing myco-heterotroph research, because of the major obstacles to measuring the fitness of filamentous fungi under natural conditions (Pringle & Taylor, 2002).

Other questions that have received considerable attention of late, but are far from resolved, concern the trophic activities of the fungi and associated full or partial myco-heterotrophs. Studies of stable isotopes show matching ^{15}N and ^{13}C patterns between particular myco-heterotrophs and their fungi (Trudell *et al.*, 2003), and that photosynthetic orchids of the forest, and even grassland, acquire carbon and nitrogen from their mycorrhizal fungi (Gebauer & Meyer, 2003). However, the quantities and dynamics of carbon gain via fungi remain to be fully characterized in any partial or full myco-heterotroph. To adequately assess the relationship between myco-heterotrophy and specificity, measurements of both carbon dynamics and specificity in a large number of species will be needed to identify trends that stand out against the idiosyncratic evolutionary history of any particular species. Further breakthroughs have included the demonstrations that certain *Rhizoctonia* species belonging to clades within the Sebacinaceae and Tulasnellaceae form full-fledged, and in some cases abundant, ectomycorrhizae on autotrophic hosts surrounding particular myco-heterotrophs (Selosse *et al.*, 2002a; Bidartondo *et al.*, 2003). But the trophic activities of most orchid-associated *Rhizoctonia* species remain obscure.

Are these questions worthy of the considerable research effort they imply? While myco-heterotrophs may not be dominant components of terrestrial ecosystems, they offer important model study systems in at least two respects. First, it is now clear that even 'normal' photosynthetic plants may rob carbon from one another via mycorrhizal fungi (Simard *et al.*, 1997). Because of the unidirectional net flow of carbon and high specificity in myco-heterotrophs, they provide the most tractable systems with which to study mycorrhizal carbon transfer. Second, much of our understanding of the evolution of parasitism derives from a few stereotypical interactions, such as those between herbivorous insects or pathogenic fungi and their host plants. Myco–heterotrophs turn these interactions on their heads, since it is the plant that preys on the fungi. Ecological and evolutionary patterns in myco-heterotrophs that mirror those in more conventional parasites (e.g. frequent host-switches which are correlated with speciation events) will aid in identifying fundamental attributes of parasitism.

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Key words: Myco-heterotrophs, fungi, mycorrhizas, orchids, achlorophyllous/nonphotosynthetic plants.

Meetings

The CO_2 fertilising effect – does it occur in the real world?

The International Free Air CO_2 Enrichment (FACE) Workshop: Short- and long-term effects of elevated atmospheric CO_2 on managed ecosystems, Ascona, Switzerland, March 2004

It would seem simple. There are only two immediate primary responses of plants exposed to elevated levels of atmospheric CO_2 concentration above the ambient (which currently averages approx. 375 ppm by volume, 33% up from the preindustrial 280 ppmv). First, in C_3 species, competition between CO_2 and O_2 at the active site of the photosynthetic enzyme 'rubisco' is shifted in favour of reaction with CO_2 thereby increasing gross photosynthetic CO_2 fixation and decreasing CO_2 loss via photorespiration. Second, in most species, both C_3 and C_4, stomatal aperture narrows thereby reducing stomatal conductance and, combined with the photosynthetic response, leads to increased water use efficiency in C-acquisition. That's it. No other primary responses have been identified, although I do wonder about whether there are subtle developmental effects associated with interactions between endogenous ethylene production and action and atmospheric CO_2 concentration. But nothing in nature is simple and, with there being two known primary responses, the long-term repercussions for ecosystems may be twice as difficult to quantify as the linked issue of impact of the increasing greenhouse gas concentration on global climate for which there is only one primary response – namely, more of the infrared back radiation emitted from the Earth's surface is absorbed in the lower atmosphere.

'Should this be seen as a mechanism of plants "resisting" a positive response to elevated CO_2, i.e. showing resilience to change?'

Box 1 FACE methodology

- FACE methodology (Lewin *et al*., 1992; Hendrey *et al*., 1999; Miglietta *et al*., 2001; Okada *et al*., 2001) involves a ring of separately controlled CO_2 release points above the ground in circles from 1 m to 30 m in diameter.
- The point-releases can be computer-controlled to be always upwind of the central experimental zone (or 'sweet-spot'), with the rate of release varied more or less with windspeed.
- There have been 13 large diameter-ring (> 8 m) FACE systems in the world, 10 still operating.
- There are approx. 20 'miniFACE' ring systems 1–2 m in diameter, for which CO_2 is usually released all around the ring continuously by day. The small ones do not have scope for a wide guard-zone around the sweet-zone and are unsuited to tall vegetation.

For both the 'greenhouse' and 'CO_2 fertilising' effects, debate has persisted over at least half a century as to whether these primary effects are leading, respectively, to global warming, and to increased vegetation productivity and C-stocks in the terrestrial biosphere. In both debates the power of constraints and feedbacks (both negative and positive) developing through time, and operating on various timescales and spatial scales, in the complex, adaptive climatic and ecological systems, have been invoked by some to argue for resilience to change. In both cases the debate continues despite continuing accumulation of observational evidence. For the CO_2 fertilising effect, both new evidence and continuing debate was seen at the recent Free Air CO_2 Enrichment (FACE) workshop in Switzerland (http://face2004.ethz.ch/index.htm). How resilient are plant processes, crop yields, ecosystems and the terrestrial C-cycle to modification by elevated atmospheric CO_2 concentration in the long term?

Doubts about long-term field-expression of the CO_2 fertilising effect arise partly because the majority of such research has been in chambers, glasshouses, open-topped chambers, and controlled environments of various types, these often being short-term experiments. However, the longest enrichment experiment by far has been in open topped chambers on a salt marsh vegetation on Chesapeake Bay. Bert Drake (Smithsonian Environmental Research Center, Edgewater, MD, USA) reported at the meeting that after 17 yr the elevated CO_2 concentration had increased the marsh shoot density by > 100% compared with ambient air control chambers. The development of the FACE technology (Box 1) in the mid-1980s by George Hendrey at the Brookhaven National Laboratory (Upton, NY, USA) has provided the opportunity to test responses in the field.

FACE versus Chamber – are the minor differences real?

Kimball *et al*. (2002) conducted a quantitative comparison of the conclusions about elevated CO_2 effects on 11 crops (including grass, cereal, C_4 sorghum, tuber and woody crops) from the four FACE experiments then available, compared with results from the large number of prior chamber experiments (including open-topped field chambers) over many years. It was comforting to those using both kinds of facility that FACE experiments had, with two exceptions and within the ranges of variability of reported results, confirmed under longer-term field conditions all the prior quantitative chamber-findings on crops grown and measured in elevated CO_2 concentration compared with ambient CO_2 concentration (persistently increased light saturated photosynthesis, decreased stomatal conductance, decreased water use, increased shoot biomass growth, increased root growth, decreased specific leaf area, decreased leaf nitrogen concentration, increased soluble carbohydrate content, little effect on phenology, and increased agricultural yield though for small grain cereal yield the increases were at the lower end of the range found in enclosed environments). The two exceptions were for reduction of stomatal conductance and enhancement of root growth relative to shoot growth, both of which were more strongly expressed in the FACE experiments than in the chamber experiments.

Lisa Ainsworth reported results of a statistical meta-analysis of results now available from experiments conducted over several years in 12 large-scale FACE facilities on four continents (Long *et al*., 2004). This again confirmed, with greater statistical rigour and for a much wider range of species including crops, pasture species and trees, most of the conclusions of the evaluation by Kimball *et al*. (2002) for a CO_2 concentration of 550 ppmv. In addition, she noted that, in the open field, elevated CO_2 increased apparent quantum yield of light-limited photosynthesis by 13% (a figure close to the theoretical short-term response expectation), that growth under water or N stress exacerbated the response of stomatal conductance to elevated CO_2 concentration, and agricultural yield increased by 17% (average of C_3 and C_4) a figure similar to the average of 15% (scaled to 550 ppmv CO_2) reported by Kimball (1986) for prior chamber studies. However, again the responses of rice and wheat yields were found to be lower than in chamber studies. Growth rate of above-ground biomass was also increased on average across all C_3 and C_4 species by 17%. For trees it increased by 28%, though this high result is influenced by the strong positive response of fast growing poplar saplings. Dicots were more responsive than grasses, and legumes more responsive than nonlegume forbs. Interestingly, the decrease in N-content per unit leaf area that has generally been observed in elevated CO_2 chamber-studies was less pronounced in FACE experiments, −4% on average, a decline

consistent wholly with the reduction in Rubisco content. To establish whether the apparent, relatively minor, differences in results between the FACE and enclosure experiments are real, coordinated FACE and enclosure experiments are needed as Alistair Rogers observed.

Positive and negative feedback

A fast-acting negative feedback, which has often been thought might lead to lower fractional response of growth than of photosynthesis rate in the short term (days to weeks), is down-regulation of photosynthesis under continuous exposure to elevated CO_2 associated with reduced leaf N-content. Ainsworth's meta-analysis confirmed that this does usually occur in the field, with the maximum carboxylation capacity ($V_{c,max}$) decreasing on average by 13% under continuous exposure to elevated CO_2. Down-regulation of $V_{c,max}$ was more strongly expressed in grasses, shrubs and crops than in legumes and trees. Should this be seen as a mechanism of plants 'resisting' a positive response to elevated CO_2, i.e. showing resilience to change? Probably not. Stephen Long (University of Illinois, Urbana, IL, USA) presented an elegant exposition of how photosynthetic down-regulation involves an optimisation of the deployment of N from photosynthetic machinery to growth organs such that a balance between C-source and C-sinks is maintained in the plant under elevated CO_2 concentration – a response that generally increases the nitrogen use efficiency (Wolfe *et al.*, 1998).

At an ecosystem scale over years to decades, another type of adaptation to continuous elevated CO_2 concentration could be changes in plant community structure. One might reasonably hypothesise that species that are most responsive in growth to elevated CO_2 concentration would become more dominant over time thereby leading to a positive feedback. Mike Jones (Trinity College, Dublin, Ireland) described the Megarich study in which monoliths of six grasslands across Europe were exposed to FACE over 3–6 yr. Generally, under competition, occurrence of dicots was enhanced and monocots relatively suppressed by continuous elevated CO_2. And there was a significantly increased fraction of legumes in the swards (Teyssonneyre *et al.*, 2002). While the Megarich study did not include determination of N-fixation, the increased preponderance of legumes in the swards is supportive of the notion that, in the long run, elevated CO_2 concentration may cause N-fixation to entrain more atmospheric N_2 into the ecosystem, leading ultimately to fuller expression of the increased growth and standing biomass potential that the elevated CO_2 provides (Gifford, 1992). It might take several decades for such a positive feedback to build up in an ecosystem to the extent that it could be measured as increased N-stocks in the field. To date no FACE experiment has been for long enough. If such N-accumulation were eventually to occur much of it would be expected in the soil and, associated with it, more soil C.

Does elevated CO_2 concentration lead to more C accumulation in the soil?

Chris van Kessel (University of California, Davis, CA, USA) addressed this question by studying soil C accumulation in the intensely N-fertilised Swiss grassland FACE system. He concluded that over 10 yr elevated CO_2 concentration had no effect on soil C-stocks, no effect on soil microbial biomass including *Rhizobium* after an initial surge, and no effect on above ground litter decomposition. From this he posited the 'resilience hypothesis' that initial responses of soil C-cycle and N-cycle processes are short lived and that they relax back to their original stocks and rates. One mechanism for this may be the 'priming' of oxidation of some older more stable forms of soil organic matter by the input of more new easily oxidised organic matter as proposed by Marcel Hoosbeek (Wageningen University, Netherlands; Hoosbeek *et al.* (2004)). However, the artificial N-input to the Swiss FACE study was extraordinarily high (either 140 or 560 kg ha^{-1} yr^{-1} over the 10 yr). From an ancillary study at the same Swiss FACE site towards the end of the treatment decade, Paul Hill (University of Wales, Bangor, UK) observed that the greater potential for sequestration of C below ground was by the swards that had the lower N-supply. This partly agrees with a microcosm study in a controlled environment over 4 yr in which a native C_3-grass was grown in a very low-N soil (total initial N of 0.02%) under elevated CO_2 concentration with only 22–198 kg ha^{-1} yr^{-1} N supplied dilutely in the irrigation water. Over 4 yr the soil had gained 15–57% (respectively) more C with elevated CO_2 concentration than without (Lutze & Gifford, 1998). Thus it is possible that under both extremely high and extremely low N-nutrition, elevated CO_2 has no effect on soil C concentration while with intermediate N-nutrition elevated CO_2 increases soil C stocks. If so, that would parallel the tendency for plant N concentration to be unaffected by elevated CO_2 concentration at extremely low and high N-status, but diminished by elevated CO_2 concentration in the intermediate range of N-nutrition (Gifford *et al.*, 2000). Resolution of this issue is one for which long-term investigations are required. The workshop returned again and again to the need for long-term experiments in the field.

The profits and pitfalls of FACE

Every experimental system in vegetation studies has its advantages and drawbacks. The great advantage of the FACE approach is that it is technically possible – if funded appropriately – to apply long-term CO_2 treatment to patches of existing ecosystem, even tall ones, over the long-term. Also leaf temperature can respond to the reduced transpiration naturally in the open air. George Hendrey urged researchers to be more aware of several inherent limitations with the

FACE approach. He emphasised particularly the rapid (down to minutes or seconds) and sometimes large fluctuations in concentration of CO_2 at each point in a FACE-ring owing to the inherent time delays of enrichment associated with sample-line length, with distance from release point to sweet-zone, with wind speed and direction changes, and with the eddy-structure of the atmosphere on the scale of FACE rings. CO_2 concentration at any one place can undergo large fluctuations within seconds to minutes under FACE, a feature that is not mirrored, in terms of either amplitude or frequency spectrum, in the control treatment. Hendrey's analysis (Hendrey *et al.*, 1997) of the impacts of such fluctuations combined direct measurements of the fluorescence responses of wheat leaves exposed to such CO_2 fluctuations, which are embedded in the unweighted mean CO_2 concentration, concluded that photosynthesis rate can be decreased by 17% or more for the mean concentration reported when that mean is of large CO_2 fluctuations on the order of half the mean, and the deviations from the mean occur over a minute or longer. This derives from the fluctuating concentration driving the internal leaf concentration into the saturated part of the photosynthetic response curve. The larger the concentrations swing above the saturating concentration the worse the underestimate becomes of the response at the calculated mean CO_2 enrichment.

A poster by Joe Holtum and Klaus Winter showed experimental data supporting Hendrey's conclusion. They showed (Holtum & Winter, 2003) that for two tree species the photosynthetic enhancement by CO_2 concentration elevated to 600 ppmv was diminished by one third when that concentration was an average of subminute fluctuations between 433 and 766 ppmv. They also reported that the 26% growth response of rice seedlings to a stable 600 ppmv CO_2 was eliminated when that average comprised 30 sec fluctuations having just a 150 ppmv amplitude. Thus extant FACE technology might be systematically understating the effect of globally elevated CO_2 on ecosystem productivity. However, it is not only FACE facilities that can suffer such fluctuations. Open topped chambers and poorly designed or managed enrichment systems in CO_2-enriched growth-chambers can also produce large 'hunting' effects that the investigators may be unaware of.

Thus CO_2 concentration fluctuations in CO_2 enriched but not ambient treatments may be a more general problem for elevated CO_2 plant research than even Hendrey and Holtum realised. In chambers, however, it should not be such an insurmountable problem as in FACE. Perhaps a 'second-best' way forward is to routinely characterise the fluctuations and to model the effective concentration that the plants perceive. That would require, however, clear understanding of all the mechanisms involved. There might be other mechanisms. For example, regular fluctuation of CO_2 concentration on a 10–30 min timescale might resonate with the inherent relaxation time of stomatal opening or closing and sometimes drive the pores fully open or fully closed artificially.

A second major potential problem for FACE technology is ethylene contamination of the CO_2. Carbon dioxide sources vary enormously in their level of trace ethylene. Supplier scrubbing methods may be of variable efficacy. In our hands even when the supplier's quality control laboratories indicate virtually undetectable levels, our own routine ethylene scrubbing columns (containing proprietary potassium permanganate-based oxidation granules) can change colour at considerably different speeds from batch to batch of CO_2 gas delivered. Ethylene scrubbing has been a substantial cost for growth chambers studies in my laboratory since identifying the problem with our supplies (Morison & Gifford, 1984). For FACE, the huge quantities of gas used might preclude routine on-site scrubbing. Ethylene, being a natural plant hormone, has growth inhibitory and specific developmental effects on some, but not all, species in the part per billion range. Apparently this is a problem that no FACE, and not all chamber, investigators have addressed in the past. As with the fluctuating CO_2 concentration issue, the implication is that the methodology may understate the productivity-enhancing effect of elevated CO_2. However, in some chambers having low air replacement rates, there is the added problem that ethylene naturally produced by the plants themselves can build up to inhibitory levels (Klassen & Bugbee, 2002). As CO_2 and ethylene interact physiologically (at the higher CO_2 levels involved in fruit ripening research, at least) this may also produce subtle confounding interactions in some chamber studies too.

Perspectives

In summary, as with global warming, there are substantial issues yet to be addressed with the CO_2 fertilising effect, but the evidence for its existence in the real world continues to consolidate. Long-term FACE studies are showing that the CO_2 fertilising effect on vegetation productivity may not, after all, be an artefact of 'plant physiologists and their greenhouses'.

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