



Challenges for mycorrhizal research into the new millennium

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Abstract

Some future directions for mycorrhizal research are discussed. Data from The IPCC Third Assessment Reports 2001 on elevated carbon dioxide the concentration and consequences regarding global climate change, form the basis for arguments that a better understanding of the mycorrhizal activities with regard to controlling carbon flow and plant nutrient flow is essential. Mycorrhiza can be regarded as energy and nutrient switches in the plant roots and it is suggested that more studies on mycorrhizal carbon relations are necessary. The impact of the genomic research era on mycorrhizal research is suggested to be of major importance during the years to come, and it is claimed that stronger interactions between different disciplines will be essential.

Introduction

The importance of mycorrhizal symbioses in nature cannot be overestimated. The significance of these interactions in the nutrition and well-being of the individual plant partner is well established (Smith and Read, 1997) and recent evidence also indicates that they may also have major effects on the structure of the plant community (van der Heijden et al., 1998). Their ubiquitous existence and impact on plant and soil biology make it necessary to consider them in all terrestrial ecological research. Since this form of symbiosis was first described in the mid 19th century, the fundamental questions surrounding these intriguing biological systems have attracted a large number of researchers, as has the question regarding potential applications in agriculture and forestry to enhance plant production and sustainability. Most work has been carried out on the ectomycorrhizal and arbuscular mycorrhizal systems, a fact which is not surprising considering their dominance in nature.

It has been predicted that the 21st century will become the century of biology as the 20th century was the century of physics, the so called 'new biology' being the justification for such a prediction. Bearing in mind the critical importance of the mycorrhizal symbioses in nature and their strong influence on many ecological processes, mycorrhizal researchers are in a very favourable position to develop the field of my-

corrhizal research even further. This paper exemplifies some areas of research which have the potential for exciting and important development, and some of the prerequisites for such development are discussed.

The mycorrhizal research community

Mycorrhizal researchers have traditionally been recruited from a number of established disciplines such as botany, microbiology or ecology. This is not surprising when one considers that mycorrhizal symbiosis in itself is a very integral phenomenon. For the advancement of our knowledge and understanding of these complex systems, the international meetings dedicated to the study of mycorrhizas have undoubtedly been of great importance. During the past 20 years, these meetings have grown considerably. Based on the number of abstracts submitted to the international conferences with mycorrhiza as the main theme, it seems that the number of researchers involved in mycorrhizal investigations has increased four times the past 20 years. The North American Conferences on Mycorrhiza (NACOM) had a strong international participation, and in 1979 the 4th NACOM attracted 106 abstracts. Since International Conferences of Mycorrhizas have been organized (ICOM, 1996, 1998, 2001), they have attracted between 400 and 500 contributions and the NACOMs and the

European Symposia on Mycorrhiza (ESM) in the late 1980s and early 1990s each attracted 200–250 abstracts. Compared with other comparable fields of research, this seems to represent normal growth, and mirrors the general increase in societies' scientific effort rather than the specific growth of the mycorrhizal research community. The mycorrhizal research community thus does not seem to have attracted more than the normal number of new researchers in spite of the growing acceptance of the biological importance of such symbioses.

A quick glance at the titles of invited presentations and abstracts submitted to the mycorrhizal conferences (NACOM, ESM and ICOM) indicates that the interest in ectomycorrhiza and arbuscular mycorrhiza over time has varied somewhat and considerably more abstracts were submitted to the last three NACOMs (1987, 1990, 1993) dealing with arbuscular mycorrhizas compared with abstracts on ectomycorrhizal studies. The number of ectomycorrhiza related abstracts has generally been larger at the European meetings (ESM). Fewer AM abstracts were submitted to the first ICOM (1996) while in 1998 the numbers were similar. The number of contributions dealing with other types of mycorrhiza, such as orchid and ericoid mycorrhizas, is consistently very low. Studies describing applications of mycorrhiza in agriculture and forestry tend to account for 20% of all abstracts, while contributions describing the development of new experimental techniques only constitute a few percent. All the symposia on mycorrhiza seem to be very strongly specialised; there have been very few contributions on more general biological subjects related to mycorrhiza research.

Global climate change, carbon and mycorrhiza

During the past 100 years we have seen continuous increase in the carbon dioxide concentration in the atmosphere, as well as an increase in mean temperature. Data on this, as well as predicted future scenarios, are convincingly presented in the IPCC (Intergovernmental Panel on Climate Change) Third Assessment Report — Climate Change 2001 (<http://www.ipcc.ch>). From the beginning of the 19th century to today, the carbon dioxide concentration has increased from 280 to 360 ppm, a concentration that has not been exceeded during the past 420 000 years and probably not during the past 4 million years. There are today overwhelming arguments that human activity is the main

cause for this increase. Seven different but realistic scenarios have been tested by the IPCC working group for the prediction of future carbon dioxide concentrations and these suggest an increase in atmospheric carbon dioxide concentration during the coming 100 years from today's value of 360 ppm to between 550 and 950 ppm. Other greenhouse gases such as methane and nitrous oxide have also been modelled. All readings of the earth's surface temperature show that the average global temperature has increased by about 0.6 °C (± 0.2 °C) over the past 100 years, and it is likely that the 1990s was the warmest decade since recording started. The relationship between the increase in the concentration of greenhouse gases and temperature increase is generally accepted. In the IPCC reports future temperature increases are also modelled according to different scenarios. These models indicate that the expected increase in the Earth's surface temperature will be between 1.4 and 5.8 °C by the year 2100. The predicted climate changes resulting from such a temperature increase are also discussed in the IPCC reports, as well as possible stabilization scenarios.

Scenarios such as those mentioned above were one basis for the Kyoto Protocol to the United Nations Framework Convention on Climate Change in 1997 which, during the summer of 2001 was agreed upon after negotiations in Köln by the majority of the signing members. In this protocol carbon dioxide problems play a very important role and each of the signatories commits itself not only to following agreed CO₂ emission reductions and/or increased sinks, but also to promoting research on carbon cycling, carbon budgeting, etc. Unfortunately, a few major industrial countries have declared that they will not sign the treaty. This, however, does not make it less important.

Of major importance for our understanding of carbon cycling is of course, that reliable carbon budgets can be determined. Much effort has already been devoted to this already during the international IBP and IGBP programmes during the 1970s and 1980s. However, fundamental information is still lacking (Schimel, 1995), including reliable estimates of terrestrial carbon pools and flows to and from these pools. These flows are partly regulated by mycorrhizas, and further research into carbon relations in mycorrhizal symbioses may turn out to be crucial in developing reliable terrestrial carbon budgets and carbon cycling models.

From microcosm studies we know that in ectomycorrhizal symbiosis, 20–30% of the carbon assimilated by the host plant may be consumed by the fungal part-

ner (Bidartondo et al., 2001; Ek, 1997; Rygielwicz and Anderson, 1994; Söderström and Read, 1987), and similar figures have been found in arbuscular mycorrhizal systems (Jakobsen, 1995; Jakobsen and Rosendahl, 1990). These estimates were recently confirmed in an elegant field study, in which large-scale girdling of mature pine trees was carried out and the reduction in soil respiration was used to estimate the contributions from saprophytic processes, root respiration and mycorrhizal respiration (Högberg et al., 2001). It thus seems clear that a substantial proportion of the carbon assimilated by the mycorrhizal host plants goes straight into the soil where part of it is respired to produce energy for biomass build-up, and part of it will be found in fungal biomass in the soil carbon pool.

Mycorrhizal fungi may also more directly control the primary production in that they are the major nutrient scavengers for their host plants (Smith and Read, 1997). However, it does not appear that the size of the plant's energy investment in its fungal partner is related to its nutritional benefits. Smith et al. (2000) showed that a fungus (*Scutellospora calospora*) which formed more biomass and thus consumed more carbon was not as efficient in phosphorus transfer to its host plant as was another fungus (*Glomus calledonium*), which produced considerably less biomass when they were both symbiotic with *Medicago truncatula*. *S. calospora* produced almost twice as much biomass while the phosphorus transfer to the host plant was almost three times greater than with *G. calledonium*. Similarly, Bidartondo et al. (2001) showed that of the four ectomycorrhizal fungi they tested, the isolate that consumed less of the host's assimilated carbon was the one that transferred most ammonium to its host when this nutrient was added to the mycelium. To make the situation even more complex, they also showed in the same experiment that of the four fungi studied, *Paxillus involutus* produced the lowest amount of mycorrhizal connections to its host plant, but was able to extract more carbon from its host per mycorrhizal connection, and also transferred most ammonium per connection. Furthermore, this fungus respired less per unit biomass than did the other fungi. The poor relation between the fungal carbon demand and nutrient supply to the host plant may initiate discussions on possible manipulation of fungi and primary production.

From the examples given above, it is easy to compare mycorrhizal fungi to carbon switches situated in the plant roots with the potential to switch the carbon

flow to the soil, where it may accumulate or be oxidized to carbon dioxide, as well as the nutrient flow to the host plant and thereby indirectly controlling the carbon dioxide assimilation. This strongly emphasizes the importance of further studying the carbon relations of mycorrhiza. In a recent review on mycorrhizal fungi and elevated carbon dioxide concentrations, Treseder and Allen (2000) concluded: "Our knowledge indicates that the influence of mycorrhizal fungi on carbon dynamics under global change remains largely unknown, but could be a significant factor in soil carbon sequestration". A challenge for mycorrhizal research is thus to gain a much better understanding of carbon flows in mycorrhizas and ways to control these, and to relate this understanding to research on ecosystem carbon budgets which, in turn, are fundamental for carbon dioxide balance on a global scale. The predicted temperature increase of perhaps as much as 5 °C in the next 100 years is a major threat to our planet.

Mycorrhiza and genomic biology

During the past decade much effort and money have been invested in genome-related research. The launch and successful realization of the HUGO (human genome) project and the subsequent sequencing of the genomes of a range of other organisms have totally changed the foundation on which much biology research is based. Today, the complete genomes of, for example, more than 30 bacterial species, *Caenorhabditis elegans*, *Drosophila*, rat and *Arabidopsis thaliana* have been sequenced, and more organisms are being added to the list. This knowledge will have important, and perhaps even revolutionary, consequences for biological research in the years to come. We also see sequencing facilities becoming common in many research organisations. DNA sequencing is still an expensive task, but the cost has decreased considerably and there is good reason to believe prices will decrease even further. Most of the sequences are, furthermore, freely available on the Internet, an enormous source of information most biologists will have to learn to use. However, the means with which to handle and analyse all this information, bioinformatics, is still under development, but will hopefully soon be easily managed by non-specialists. As an effect of these extensive sequencing programmes we now see the development of functional genomics, transcriptomics, proteomics, metabolomics, etc., sub-disciplines which can all be included in the concept of genomics. What all these

new terms illustrate is the extremely rapid development within this field of science, a development which is partly an effect of the many man-hours being invested, but also an effect of novelties being brought out into the open. It is a challenge to every biologist today to learn how to use this new information for his or her particular purposes.

Genome-related research has much potential earlier not evident to biological research. Because of the extensive technological development within this area, there is potential for what may be called 'industrial research', i.e., the potential to run large-scale experiments and analyses since much of the analyses can be automatized. By analysing traditional problems from a genomic perspective, physiological or ecological questions can be elucidated in a new way. It may also be possible to formulate new problems from a genomic analysis, problems that may find physiological or ecological solutions. In fact we may soon also be able to study gene expressions in nature to a much greater extent than has hitherto been possible. The methods being developed today may allow us to perform complex observational and experimental analyses in nature and may also allow new types of experiments to be designed.

One very interesting effect of the growth of genomic biology is that within the broadly defined subject of bioinformatics, new and very promising cooperation between biologists, applied mathematicians and computer scientists has been developed. New courses and educational programmes in computational biology, theoretical biology and bioinformatics are being established at most institutes of higher education all over the world, programmes in which students in biology, mathematics and computer science can meet and work on common projects. Given the complexity of biological systems, this kind of cooperation is necessary if we are to model these systems. This development may well open up new research areas in biology. The possible impact of genomic biology on biology in general has been discussed in a number of reviews, e.g. by Brent (2000).

Genomic biology has already had an important influence on mycorrhizal research, something which is very obvious when studying the programme for ICOM3. Without even attempting a review of genome related mycorrhizal literature, a few examples will be mentioned. The best established methods making use of genome-related molecular biology are those used for identification/classification of mycorrhizal fungi, methods that have almost become routine in many

laboratories. The most frequently used technique is PCR-RFLP of the nuclear ribosomal DNA ITS region, and species libraries have been established at many laboratories. It should soon also be possible to identify any fungus to species level by use of only vegetative samples and when PCR-RFLP fails DNA sequencing will be an option. By applying PCR-RFLP it has now been well established, contrary to what was earlier believed, that the formation of fruit bodies is not a good reflection of the species composition and species frequency of the ectomycorrhiza community structure in any ecosystem (Buscot et al., 2000; Erland and Taylor, 1999). Instead it seems that a few species are very common and widely spread, some of which were almost never encountered in fruit body inventories.

Gene expression in mycorrhizal systems has been studied for some 10 years and these studies have recently been reviewed (e.g., Harrison, 1999; Lapopin and Franken, 2001; Martin and Tagu, 1999; Martin et al., 1999; Tagu et al., 2000). However, until now, it has not been possible to study more than one or a few genes simultaneously. Considering the complexity of the symbiosis, with two independent partners having to establish themselves separately, and then, as symbiotic partners, each reacting to environmental stimuli, etc., it is clearly disadvantageous to be restricted to single-gene studies. With the advent of sequencing facilities and the progress made in the large scale genome sequencing programmes, completely new opportunities are now available for global expression analysis using cDNA libraries for microarray analysis. The potential of this method has been widely discussed (e.g., Brown and Botstein, 1999; Harrington et al., 2000; Richmond and Summerville, 2000) and the first results obtained by applying these methods have been published (e.g., yeast: Jia et al., 2000; *Arabidopsis*: Schaffer et al., 2001). An attempt to apply these methods for classification in cancer diagnosis (Kahn et al., 2001) resulted in an editorial comment entitled 'Microarrays — the 21st century divining rod' (He and Friend, 2001). The large-scale gene expression analysis which is today within reach via mRNA profiling and bioinformatics may have an immense impact on research on all complex biological systems, not least the mycorrhizal system.

The first global gene expression data from mycorrhizal ecosystems have recently been published. Voiblet et al. (2001) presented results of an analysis of symbiosis-related genes in *Eucalyptus globus* – *Pisolithus tinctorius* ectomycorrhiza while Johansson et al. (2001) studied *Betula pendula* – *Paxillus involutus*

ectomycorrhiza. The first authors performed a more detailed analysis of the differential expression of 486 selected genes in the fungus, host plant and mycorrhiza, respectively, while the latter authors reported a number of differences in the first functional profiling of the 1900 putatively unique transcripts from the fungus, plant and mycorrhiza. Both these papers describe the first steps towards the possible microarray analysis of the systems in order to describe the gene expression during formation of the symbiosis, as indicated by Martin (2001).

At present, progress in genomic research is extraordinarily fast, and we can still only speculate on the consequences of the extensive sequencing programmes in progress. However, putting this progress to good use presents a major challenge for mycorrhizal researches, as discussed in more detail by Martin (2001) and Franken and Requena (2001). As Martin (2001) points out: "While familiar with the practicalities of making a PCR or a biochemical analysis, many scientists lack a 'nuts-and-bolts' appreciation of the pros and cons of functional genomics". This is a major challenge for mycorrhizal research, to get into the fast lane of functional genomics, and not to be left behind in the fast progress of this expanding field.

The total mycorrhiza research effort

Mycorrhizal symbiotic systems are not only of the utmost ecological importance, for agricultural and forestry production, but also for the sustainability of these and of natural ecosystems. As discussed above, they may also be of primary importance for the global carbon balance which influences climate change. However, they also form superb model systems for studies of interactions between organisms. Awareness of the importance of mycorrhizal symbioses has increased. However, as indicated earlier in this paper it does not seem that the mycorrhizal research community has increased relative to other fields.

Literature databases can be used to obtain an indication of the relative volume of research. A search in the BIOSIS database reveals that the number of articles from 1993 to spring 2001 on 'mycorrhiza' is 4600, and in Figure 1A it can be seen that this is almost the same as the number of papers published on, e.g. *Rhizobium* (incl. *Bradyrhizobium*) or *Agrobacterium*. All publications on nitrogen fixation (incl. *Rhizobium*) amount to 7500. The total volume of research on my-

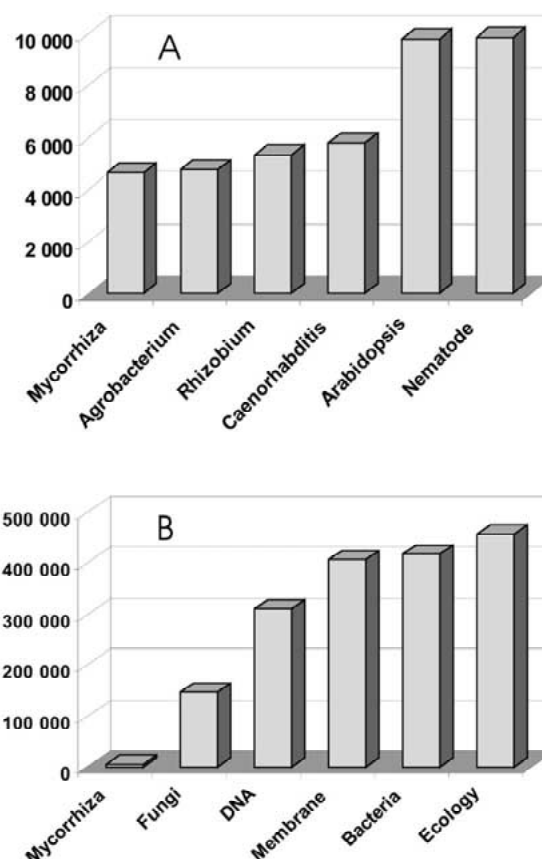


Figure 1. (A,B) Numbers of publications between 1993 and April 2001 in different subject areas, extracted from the BIOSIS database.

corrhiza thus seems to be equal to that on *Rhizobium*, the most important nitrogen-fixing bacteria (very few papers are published on other nitrogen fixing systems) but is considerably less than the total nitrogen fixation volume. Papers on the use of *Agrobacterium* as a plant transgenic agent (which is probably the major share of the articles with the word '*Agrobacterium*') are equal to mycorrhiza in numbers. The volume of research on *Arabidopsis*, the first plant for which the complete genome was sequenced, and one of the general genetic model organisms, is about twice as large as that on all mycorrhizal research.

A search on more unspecific subjects naturally results in many more publications. Searching on a word like 'fungi' results in 146 000 articles (Figure 1B), indicating that the total volume of research on this group of organisms is about 30 times larger than that on mycorrhiza. Searching for a word like 'membrane' will give 400 000 articles and results from some other wider areas are also shown in Figure 1B.

Considering the breadth of the research in mycorrhiza, as is well illustrated by the abstracts from the conferences discussed above, the volume of mycorrhizal research seems small compared with other more specialised areas of research. However, at the same time it appears that contributions on mycorrhizal research are sparse at other symposia of obvious relevance and it thus seems that reports on the most current advances in mycorrhiza research are not communicated as efficiently as one might expect. In the Proceedings of The 8th International Symposium on Microbial Ecology, Halifax, 2000 (Bell et al., 2000) only 10 pages out of 934 dealt with mycorrhiza. Another example is the XXI International Fungal Genetics Meeting in California in 2001: of the 502 abstracts in the abstract book, only five were mycorrhiza related. Similar figures can be found for botany and plant physiology. A major challenge for mycorrhizal researchers in the years to come will thus be to place mycorrhizal symbiosis more centrally in the expanding research efforts in biology.

Conclusions

Global climate change is of great concern and is of fundamental importance for our planet. Since mycorrhiza may play a critical role in terrestrial carbon exchange processes, a deeper understanding of these processes is urgently needed.

As a consequence of the fast and revolutionary development in genomic and postgenomic research, extraordinary progress in understanding complex biological systems can be expected in the years to come. It is imperative to include this development in all long-term biological research planning. This calls for strong interactions between different disciplines of science.

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