New perspectives towards analysing fungal communities in terrestrial environments

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Fungi play key roles in numerous ecosystem functions, and recent advances in the study of fungal diversity and ecology have led to a greater appreciation of this group of microeukaryotes. The application of a variety of nucleic acid techniques to fungal classification and phylogeny has led to a number of evolutionary insights, and has also begun to provide the necessary information for identification of unknown isolates and DNA sequences. These data, together with direct molecular characterisation of fungi in the field, provide new possibilities to describe fungal diversity and distribution. Such advances will no doubt also provide the means for a more detailed interpretation of ecological experiments.

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Abbreviations

AMF	arbuscular mycorrhizal fungi
ITS	internal transcribed spacer
PLFA	phospholipid ester-linked fatty acid

Introduction

Fungi do not only manifest themselves above ground, but also profoundly effect below-ground processes, including decomposition, plant pathogenicity, disease suppression, and nutrient uptake. In spite of their documented role in ecosystem functioning, little is known about their population dynamics, community structure, and diversity. Difficulties encountered with the identification, isolation, and quantification of many fungi, especially in complex environments, has hampered efforts to fill this informational void. Molecular biological techniques, especially nucleic-acid-based strategies targeting rRNA-encoding regions, present new possibilities for understanding the nature and role of fungi in terrestrial ecosystems.

Much of the recent progress in the description of fungi in terrestrial ecosystems has stemmed from the need to detect and track organisms with large economic impacts, for example, crop pathogens and beneficials. Such studies not only augment the mycologist's arsenal of molecular techniques, but also expand the comparative molecular database for future studies. Focus on specific strains of interest, however, has not necessarily led to many novel conceptual insights concerning the ecology and diversity of fungal communities in general. Nonetheless, elegant experimental data continue to accumulate regarding the range of fungal functions and interactions within terrestrial communities. This article will review some of the recent technological and informational advances in the molecular detection and description of fungi, and address how the use of such techniques can lend new perspectives toward understanding fungal ecology in terrestrial ecosystems.

Taxonomy and phylogeny

The comparison of nucleotide sequences, especially of rRNA genes, has revolutionised microbial phylogenetics and taxonomy over the past two decades [1]. The recovery of sequence information directly from the environment has provided a means of characterising organisms without the prerequisite of culturing them [2,3]. Similarly, molecular data provide a means of distinguishing between organisms lacking sufficient morphological identifiers. The use of such DNA markers was thought ideal for the study of prokaryotes, as many remain recalcitrant to laboratory culture and display few useful taxonomic characters. This holds true of many fungi, especially those with intimate associations with plants or other organisms. Culture-based studies often fail to describe accurately fungal populations for several reasons. These include the common existence of multiple life-cycle morphologies, differential spore germination and hyphal propagation on plating media, and the lack of reliable distinguishing characters for some taxa.

White *et al.* [4] pioneered the use of rRNA markers in the study of fungi with the introduction of PCR methods for the amplification of regions of the nuclear and mitochondrial rRNA operons. These authors realised the utility of both relatively well-conserved markers (small-subunit rDNA) for phylogenetic comparison across broad taxonomic levels, and rapidly evolving markers for fine-scale differentiation (ribosomal internal transcribed spacers [ITSs]). The ITS regions, although transcribed, do not code for any function and are therefore free to accumulate neutral mutations, making them ideal targets to discriminate between closely related organisms. To illustrate the explosion of data generated by comparative sequencing studies, White *et al.* [4] designed their primers using an alignment of three DNA sequences — the Ribosomal Database Project [5] now totals approximately 500 fungal nuclear small-subunit rDNA (18S rDNA) sequences. Sequence divergence between fungi has been used to distinguish between closely related strains [6] as well as to hypothesise ancient evolutionary divergence [7–9].

Particularly useful to future studies is the systematic accumulation and organisation of sequence information. Thus, the Ribosomal Database Project provides an invaluable tool, and other efforts to database common molecular markers should prove extremely helpful [10••,11••].

This review cannot describe all the individual advances achieved using comparative sequence analysis to study the phylogeny and taxonomy of fungi, but two good illustrations come from Dong *et al.* [12•] and Bago *et al.* [13•]. The former describes the phylogenetic analysis of nuclear rDNA sequences of both the large and small subunit-encoding genes to resolve relationships between members of the *Loculoascomycete*. The latter uses a combined analysis of 18S rDNA sequences, isozyme profiles of malate dehydrogenase, and morphological characters to identify groupings within the genus *Gigaspora*.

As the Bago *et al.* [13•] study demonstrates, nucleotide sequence comparison is not the only molecular criterion useful for phylogenetic analysis and/or taxonomic discrimination. Other strategies include: firstly, restriction fragment length polymorphism (RFLP) of PCR-amplified ITS regions [14•], including sequence-specific separation of fragments using the dye HA-Yellow [15]; secondly, reverse dot blot hybridisation [16•]; thirdly, amplification of microsatellite regions [17], also used in combination with random amplified polymorphic DNA (RAPD) markers [18••]; fourthly, amplified fragment length polymorphism (AFLP) [19,20]; and finally, universally-primed PCR fingerprinting combined with cross hybridisation [21].

Specific detection

While these methods are of interest to the taxonomist, they are also useful for detection of particular fungi in the field. Pathogens, especially plant pathogens in terrestrial ecosystems, are of key economic and environmental importance. Rapid and accurate detection of plant pathogens is often vital to the timely implementation of proper measures for crop or vegetation protection. Detection methods based upon cultivation or specific antibody screening are sometimes too slow, non-specific, or unreliable for efficient use. In such cases, highly species-, subspecies- or strain-specific nucleic acid sequences can act as targets for diagnostic PCR or hybridisation analyses [22]. Although several possibilities exist, the current targets of choice remain the highly variable ITS regions and DNA sequences derived from unique random amplified polymorphic DNA bands [23].

Recently developed assays for the specific detection of plant-pathogenic fungi are too numerous to list, but the following deserve particular mention. Bago *et al.* [24•] demonstrated the potential use of *in situ* PCR using fluorescently labelled primers to detect and localise arbuscular mycorrhizal infections. This is of particular importance as it is not only the species composition of the microbial community that is important to its functioning but also the spatial distribution of interacting organisms [25]. Thus, although the application of this in *situ* PCR technique may be limited by methodological constraints, it provides a new means of investigating the ecology of arbuscular endormycorrhizal fungi (AMF).

Disease symptoms, and loss of plant productivity, are often only seen above a certain threshold of pathogen density. Strategies that seek to quantify the relative amount of fungal material present are therefore of particular interest. Nicholson *et al.* [26] employed a competitive PCR strategy for the discrimination and quantification of cereal eyespot fungi; whereas Herdina *et al.* [27] used a slot-blot assay to estimate the amount of the causal agent of take-all disease, *Graeumannomyces graminis* var. *tritici*, in soils. The former technique has the advantage of being highly sensitive, whereas the latter avoids potential biases associated with PCR techniques. PCR-based methodologies for the rapid quantification of specific target DNAs are developing extremely rapidly. New strategies, such as the use of molecular beacons [28] and the TaqMan real-time PCR detection system (Perkin Elmer-Cetus), will no doubt be employed with increased frequency for these purposes. The TaqMan system has already been developed for the analysis of *Pythium* species in sugarcane [29].

The population dynamics of plant-pathogenic fungi is further complicated by the ecological significance of different life-cycle forms for many species. The amount of fungal material (hyphae) present may be a good indicator of the degree of fungal infestation; however, the abundance of latent units of propagation (spores) may be far more indicative of future potential for disease development. The development of methods to distinguish and quantify such different life-cycle forms should enhance our understanding of fungal ecology and aid efforts to control pathogens.

Of course, non-pathogenic fungi are also important targets for study in terrestrial ecosystems. Fungi such as ecto- and endomycorrhizal species (discussed below) and antagonistic fungi [21] can have important beneficial effects on plant growth, and these effects may be highly species or even strain specific. Fungi that can exert indirect negative effects, for example, via transmission of plant viruses, might also be targets of such detection assays [6].

Ectomycorrhiza

One of the greatest benefits offered by the use of molecular methods is the ability to characterise populations that are typically associated with other organisms. Ectomycorrhizal fungi form mutualistic associations with plants in which they typically facilitate nutrient uptake while obtaining carbon from their host. Fungal mycelia can extend some distance from the point of infection and be connected to other mycorrhizal individuals *via* a common mycelium, thus providing a means for the transfer of nutrients, including carbon, between plants, which has been coined the 'wood-wide web' [30].

Whereas some ectomycorrhizal–plant relationships are highly specific and easily identified, the diversity and function of ectomycorrhizal fungi in most terrestrial environments cannot be adequately described by the simple morphological characterisation of sporocarps (i.e. fruit-bodies) [31]. Gehring

et al. [32••] demonstrated this point in a comparative analysis that assessed ectomycorrhizal community structure by use of ITS-RFLP. They showed that assessments of aboveground sporocarp diversity could differ dramatically from the direct analysis of root tips. This technique has also been combined with isozyme analysis to study ectomycorrhizal associations in Scots pine in natural humus microcosms [33].

Sequence analysis of DNA recovered from ectomycorrhizal material also provides a means of revealing the ecology of these organisms [11••]. Through the use of a combination of molecular techniques, the level of specialisation for some ectomycorrhizal fungi is beginning to be understood. This specialisation has been elegantly demonstrated for non-photosynthetic monotropes and orchids, which can be considered parasites of their ectomycorrhiza [34,35].

Endomycorrhiza

Like the ectomycorrhizal fungi, AMF have an obligate association with their plant host, and cannot, therefore, be studied in pure culture. Many past studies have grouped all AMF into one class for the sake of simplicity; however, this ignores AMF diversity and the varying impact that different AMF species or strains can have on plant growth. To date, AMF diversity has typically been studied by making taxonomic inventories of spores or by the use of catch plants. Unfortunately, both these strategies fail to describe plant–AMF community structure accurately. Whereas spore morphology can often be used by experts to classify AMF to an acceptable taxonomic level, spore production by various AMF is far from uniform and cannot be related to the plant host of origin. So-called 'catch' plants are typically chosen based on their ability to be colonised by a wide variety of AMF species. In pot experiments, such plants are typically grown in a soil sample to determine the kind of AMF infections that can arise from the extant AMF spore populations. Whereas the use of catch plants is of prime importance in the isolation and propagation of AMF for experimental studies, it is often too tedious and unrepresentative for description of AMF diversity or community structure.

Merryweather and Fitter [36•] have recently described methods for analysing AMF in plant roots. By using a statistical analysis of nine useful morphological characters (i.e. staining intensity, vesicle shape, and vesicle vacuole number and size), the authors devised a hierarchical classification system separating the six fungal morphotypes they encountered. Such in-plant identification of AMF requires considerable expertise, but was shown to be very useful in the study of seasonal and spatial variations in AMF populations [37].

New insights are also being attained in the distribution, ecology and diversity of AMF via the implementation of molecular techniques. The strategy best used to date has been the recovery and analysis of AMF rDNA sequences from roots using PCR [38]. Helgason *et al.* [39••] used this approach to demonstrate a higher AMF sequence diversity at woodland sites in comparison to various arable fields. These authors concluded that intense agricultural land use resulted in a decline in the AMF diversity of such soils. Such studies will continue to provide new insights into AMF diversity [40].

Community analysis

An accurate description of fungal community structure cannot be achieved by simply counting and classifying fungal colony-forming units, especially in highly complex soil ecosystems. PCR-based cloning studies, while highly informative, are also often too work- and cost-intensive to allow for the description of multiple samples. Community fingerprinting techniques, such as denaturing gradient gel electrophoresis [41], in combination with primers targeting fungal rDNA, offer new strategies for the description and comparison of fungal communities [42]. Modification of this strategy to target specific fungal groups or other DNA targets should expand its use in fungal community analysis. Given the need for the quantification of specific fungal populations, the prospect of combining community analysis with competitive PCR procedures is of particular interest [43].

Signature lipids, which can be characteristic of microbial groups from the family to domain level, have also been used as markers to describe the composition of microbial communities. An example is the phospholipid ester-linked fatty acid (PLFA) 18:2ω6,9, which has been used to estimate the proportion of fungal biomass in soils [44,45]. Another useful marker in AMF studies may be the discernment of fungal biomass based upon the PLFA 16:1ω5, as demonstrated by Olsson *et al.* [46••] in a study looking at AMF growth and interactions in dune sand. Unfortunately, PLFAs have not been shown to be useful for distinguishing between different fungal taxa. Another limitation of this method involves the potentially high background PLFA levels due to interference by plant material and, to a lesser extent, bacterial lipids. As such, the application of lipid biomarkers to field studies investigating fungal communities may be somewhat limited.

Experimental data has started to shed some light on the importance of below-ground fungal diversity on terrestrial ecosystem functioning. For example, Van der Heijden *et al.* [47••] have presented compelling evidence that AMF diversity and AMF community composition are important factors in overall plant productivity and nutrient fluxes. The ability to track different AMF species and strains in such mixed inoculum experiments would no doubt provide valuable information concerning AMF competition, interaction with other organisms, and resource partitioning. Specific PCR-based tracking strategies, such as those described by Van Tuinen *et al.* [48••], show good potential in this respect. Community fingerprinting methods, as described above, also present new prospects for the culture-independent monitoring of fungal communities both in the field and during laboratory experimentation.

Conclusions

The development and use of molecular methods for the specific detection of economically important fungi will continue to increase rapidly. As molecular knowledge accrues, so too will our ability to design specific primers and probes, identify unknown isolates, and decipher evolutionary relationships. The use of multidisciplinary approaches combining several molecular techniques in combination with conventional strategies are most promising for furthering our understanding of fungal ecology in terrestrial ecosystems.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Woese CR, Fax GE: **Phylogenetic structure of the prokaryotic domain: the primary kingdoms.** *Proc Natl Acad Sci USA* 1977, **74**:5088-5090.

2. Giovannoni SJ, Britschgi TB, Moyer CL, Field KG: **Genetic diversity in Sargasso Sea bacterioplankton.** *Nature* 1990, **345**:60-63.

3. Ward DM, Weller R, Bateson MM: **16S rRNA sequences reveal numerous uncultured microorganisms in a natural community.** *Nature* 1990, **345**:63-65.

4. White TJ, Bruns T, Lee S, Taylor J: **Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics.** In *PCR Protocols.* Edited by Innins MA, Gelfand DH, Sninsky JJ, White TJ. San Diego: Academic Press; 1990:315-322.

5. Maidak BL, Cole JR, Parker CT Jr, Garrity GM, Larsen N, Li B, Lilburn TG, McCaughey MJ, Olsen GJ, Overbeek R *et al.*: **A new version of the RDP (Ribosomal Database Project).** *Nucleic Acids Res* 1999, **27**:171-173.

6. Ward E, Adams MJ: **Analysis of ribosomal DNA sequences of *Polymyxa* species and related fungi and the development of genus- and species-specific PCR primers.** *Mycol Res* 1998, **102**:965-974.

7. Bruns TD, Vigalys R, Barns SM, Gonzalea D, Hibbett DS, Lane DJ, Simon L, Stickel S, Szaro TM, Weisburg WG, Sogin ML: **Evolutionary relationships within fungi: analysis of nuclear small subunit rRNA sequences.** *Mol Phylogeny Evol* 1992, **1**:231-243.

8. Wilmotte A, van der Peer Y, Goris A, Chapelle S, de Baere R, Nelissen B, Neefs J-M, Hennebert GL, de Wachter R: **Evolutionary relationships among higher fungi inferred from small ribosomal subunit RNA sequence analysis.** *Syst Appl Microbiol* 1993, **16**:436-444.

9. Simon L, Bousquet J, Lévesque R, Lalonde M: **Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants.** *Nature* 1993, **363**:67-69.

10. •• Cullings KW, Vogler DR: **A 5.8S nuclear ribosomal RNA gene sequence database: applications to ecology and evolution.** *Mol Ecol* 1998, **7**:919-923.
The nuclear 5.8S rRNA gene is often used as a phylogenetic reference point during studies that target the internal transcribed spacer regions. Although the 5.8S gene does not contain the resolving power for intrageneric discrimination, such comparative information should prove very helpful to general placement of sequences recovered from fungal isolates and field samples. Alignment can be accessed via the World Wide Web on URL: http://userwww.sfsu.edu/~ufewkshp/58S.html.

11. •• Bruns TD, Szaro TM, Gardes M, Cullings KW, Pan JJ, Taylor DL, Horton TR, Kretzer A, Garbelotto M, Liss Y: **A sequence database for the identification of ectomycorrhizal basidomycetes by phylogenetic analysis.** *Mol Ecol* 1998, **7**:257-272.
An extensive analysis of new and old mitochondrial large subunit rRNA sequences. Alignment can be obtained or added to via the World Wide Web on URL: http://mendel.berkeley.edu/boletus.html.

12. • Dong J, Chen W, Crane JL: **Phylogenetic studies of the *Leptosphaeriaceae, Pleosporaceae* and some other *Loculoascomycetes* based upon nuclear ribosomal DNA sequences.** *Mycol Res* 1998, **102**:151-156.
A robust phylogenetic analysis that exemplifies both the possibilities and limitations of such molecular data.

13. • Bago B, Bentivenga SP, Brenac V, Dodd JC, Piché Y, Simon L: **Molecular analsyis of *Gigaspora* (Glomales, Gigasporaceae).** *New Phytol* 1998, **139**:581-588.
This paper describes a robust phylogenetic analysis and is one of the few papers to describe both the possibilities and the limitations of such molecular data.

14. • Farmer DJ, Sylvia DM: **Variation in the ribosomal DNA internal transcribed spacer of a diverse collection of ectomycorrhizal fungi.** *Mycol Res* 1998, **102**:859-865.
A good example of a restriction fragment length polymorphism study of PCR-amplified internal transcribed spacer DNA. Uses multiple restriction enzymes to produce high quality data for assessments of both inter- and intraspecific variation.

15. Fatehi J, Bridge P: **Detection of multiple rRNA-ITS regions in isolates of *Ascochyta.*** *Mycol Res* 1997, **102**:762-766.

16. • Levesque CA, Harlton CE, de Cock AWAM: **Identification of some oomycetes by reverse dot blot hybridization.** *Phytopathol* 1998, **88**:213-222.
This study uses an internal transcribed spacer probe array to allow for the rapid identification of unknown *Pythium* and *Phytophthora* isolates.

17. Longato S, Bonfante P: **Molecular identification of mycorrhizal fungi by direct amplification of microsatellite regions.** *Mycol Res* 1997, **101**:425-432.

18. •• Anderson IC, Chambers SM, Cairney JWG: **Use of molecular methods to estimate the size and distribution of mycelial individuals of the ectomycorrhizal basidiomycete *Pisolithus tinctorius.*** *Mycol Res* 1998, **102**:295-300.
This paper describes the identification and mapping of mycelial individuals. A good example of how molecular markers can be used to track individual genotypes in the environment.

19. Bonants PJM, Hagenaar-de Weerdt M, Kema GHJ, van den Boogert PHJF, Baaijen RP, Waalwijk C: **AFLP research on plant pathogenic fungi at the IPO-DLO (the Netherlands).** *Gewasbescherming* 1998, **29**:25.

20. Rosendahl S, Taylor JW: **Development of multiple genetic markers for studies of genetic variation in arbuscular mycorrhizal fungi using AFLP.** *Mol Ecol* 1997, **6**:821-829.

21. Bulat SA, Lübeck M, Mironenko N, Jensen DF, Lübeck PS: **UP-PCR analysis and ITS1 ribotyping of strains of *Trichoderma* and *Gliocladium.*** *Mycol Res* 1998, **102**:933-943.

22. Henson JM, French R: **The polymerase chain reaction and plant disease diagnosis.** *Annu Rev Phytopathol* 1993, **31**:81-109.

23. Schleier S, Voigt K, Wöstemeyer J: **RAPD-based molecular diagnosis of mixed fungal infections on oilseed rape (*Brassica napus*): evidence for genus- and species-specific sequences in the fungal genomes.** *J Phytopathol* 1997, **145**:81-87.

24. • Bago B, Piché Y, Simon L: **Fluorescently-primed *in situ* PCR in arbuscular mycorrhizas.** *Mycol Res* 1998, **102**:1540-1544.
The authors demonstrate the potential use of this new method for *in planta* detection of AMF. It is not possible to determine how well these methods can be expanded to the detection of other fungi in other plants, but the authors nevertheless make the important step of combining microscopic techniques with the use of specific molecular markers.

25. Amann R, Ludwig W, Schleifer K-H: **Phylogenetic identification and *in situ* detection of individual cells without cultivation.** *Microbiol Rev* 1995, **59**:143-169.

26. Nicholson P, Rezanoor HN, Simpson DR, Joyce D: **Differentiation and quantification of the cereal eyespot fungi *Tapesia yallundae* and *Tapesia acuformis* using a PCR assay.** *Plant Pathol* 1997, **46**:842-856.

27. Herdina H, Yang A, Ophel-Keller K: **Correlation of take-all disease severity and inoculum level of *Gaeumannomyces graminis* var. *tritici* using a slot-blot hybridization assay.** *Mycol Res* 1997, **101**:1311-1317.

28. Tyagi S, Bratu DP, Kramer FR: **Multicolor molecular beacons for allele discrimination.** *Nat Biotechnol* 1998, **16**:49-53.

29. Heelan LA, Bassam BJ, Croft BJ, Dietzgen R, Maclean DJ: **Identification and analysis of *Phythium* species in sugarcane using real time PCR detection by TaqMan™.** In *Abstracts of the 7th International Congress of Plant Pathology*. Edinburgh, Scotland; 1998:3.3.20.

30. Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R: **Net transfer of carbon between ectomycorrhizal tree species in the field.** *Nature* 1997, **388**:579-582.

31. Gardes M, Bruns TD: **Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views.** *Can J Botany* 1996, **74**:1572-1583.

32. •• Gehring CA, Theimer TC, Whitman TG, Keim P: **Ectomycorrhizal fungal community structure of Pinyon pines growing in two environmental extremes.** *Ecology* 1998, **79**:1562-1572.
This paper emphasises the need to analyse plant roots directly when assessing ectomycorrhizal community structure rather than relying on aboveground sporocarps morphology.

33. Timonen S, Tammi H, Sen R: **Characterization of the host genotype and fungal diversity in Scots pine ectomycorrhiza from natural humus microcosms using isozyme and and PCR-RFLP analyses.** *New Phytol* 1997, **135**:313-323.

34. Taylor DL, Bruns TD: **Independent, specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids.** *Proc Natl Acad Sci USA* 1997, **94**:4510-4515.

35. Cullings KW, Szaro TM, Bruns TD: **Evolution of extreme specialization within a lineage of ectomycorrhizal epiparasites.** *Nature* 1996, **379**:63-66.

36. • Merryweather J, Fitter A: **The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta* I. Diversity of fungal taxa**. *New Phytol* 1998, **138**:117-129.
The authors developed a system based upon the statistical analysis of morphological characters for the identification of AMF within roots, thus providing a means of studying AMF directly. Also used in [37].

37. Merryweather J, Fitter A: **The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta* II. Seasonal and spatial patterns of fungal populations.** *New Phytol* 1998, **138**:131-142.

38. Clapp JP, Young JPW, Merryweather JW, Fitter AH: **Diversity of fungal symbionts in arbuscular mycorrhias from a natural community.** *New Phytol* 1995, **130**:259-265.

39. •• Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW: **Ploughing up the wood-wide web?** *Nature* 1998, **384**:431.
The authors recover AMF-like rDNA from plant roots from several woodland and arable fields via specific PCR and cloning. Sequence analysis showed far greater sequence diversity in woodland sites.

40. Clapp JP, Fitter AH, Young JPW: **Ribosomal small sub-unit sequence variation within spores of an arbuscular mycorrhizal fungus, *Scutellospora* sp.** *Mol Ecol* 1999, in press.

41. Muyzer G, de Waal EC, Uitterlinden AG: **Profiling complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA.** *Appl Environ Microbiol* 1993, **59**:695-700.

42. Kowalchuk GA, Gerards S, Woldendorp JW: **Detection and characterisation of fungal infections of *Ammophila arenaria* (marram grass) roots by denaturing gradient gel electrophoresis of specifically amplified 18S rDNA.** *Appl Environ Microbiol* 1997, **63**:3858-3865.

43. Felske A, Akkermans ADL, de Vos WM: **Quantification of 16S rRNAs in complex bacterial communities by multiple competitive reverse transcription-PCR in temperature gradient gel electrophoresis fingerprints.** *Appl Environ Microbiol* 1998, **64**:4581-4587.

44. White DC, Ringelberg DB: **Utility of signature lipid biomarker analysis in determining *in situ* viable biomass, community structure, and nutritional/physiological status of the deep subsurface microbiota.** In *The Microbiology of the Terrestrial Subsurface.* Edited by Amy PS, Haldeman DL. Boca Raton, FL; CRC Press; 1995:117-134.

45. Müller MM, Kantola R, Kitunen V: **Combining sterol and fatty acid profiles for the characterization of fungi.** *Mycol Res* 1994, **98**:593-603.

46. •• Olsson PA, Francis R, Read DJ, Söderström B: **Growth of arbuscular mycorrhizal mycelium in calcareous dune sand and its interaction with other soil microorganisms as estimated by measurement of specific fatty acids.** *Plant Soil* 1998, **201**:9-16.
Both specific PLFA and neutral lipid fatty acid (NLFA) were used to estimate AMF biomass and storage material in a root-free microcosm experiment. Worked well, but may have limited field application.

47. •• van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR: **Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and production.** *Nature* 1998, **396**:69-72.
This study gives evidence supporting the notion that a greater AMF diversity can more efficiently exploit soil resource, thus leading to greater plant productivity. An important contribution to the 'diversity/ecosystem function' debate.

48. •• Van Tuinen D, Jacque E, Zhao B, Gollotte A, Gianinazzi-Pearson V: **Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR.** *Mol Ecol* 1998, **7**:879-887.
A nested PCR strategy was used to detect and discriminate between four different arbuscular mycorrhizal species in a microcosm system. It showed differential colonisation levels for the different strains, and demonstrated that a single root may have multiple infections.