SOIL MICROBIAL ECOLOGY AND PLANT ROOT INTERACTIONS

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Microorganisms are a fundamentally important component of the soil habitat where they play key roles in ecosystem functioning through controlling nutrient cycling reactions essential for maintaining soil fertility and also contributing to the genesis and maintenance of soil structure. Within the soil there exist many microbial interactions with, for example, soil invertebrates, the rhizosphere (the 3-4 mm layer of soil surrounding plant roots), mycorrhizal fungal associations and plant–pathogen relationships, and these associations contribute to the development and activity of microbial communities in soils. There is a vast abundance of microorganisms that exist within the soil, e.g., one gram of grassland soil (roughly equivalent to half a teaspoonful) typically contains about 10^9 -10^{10} bacteria (Figure 6.1) and several hundreds of metres of fungal hyphae. Whilst we have been aware of the abundance of bacteria and fungi in soils for some considerable time, it is only recently that microbial ecologists have started to appreciate their enormous diversity through the application of techniques in molecular biology.

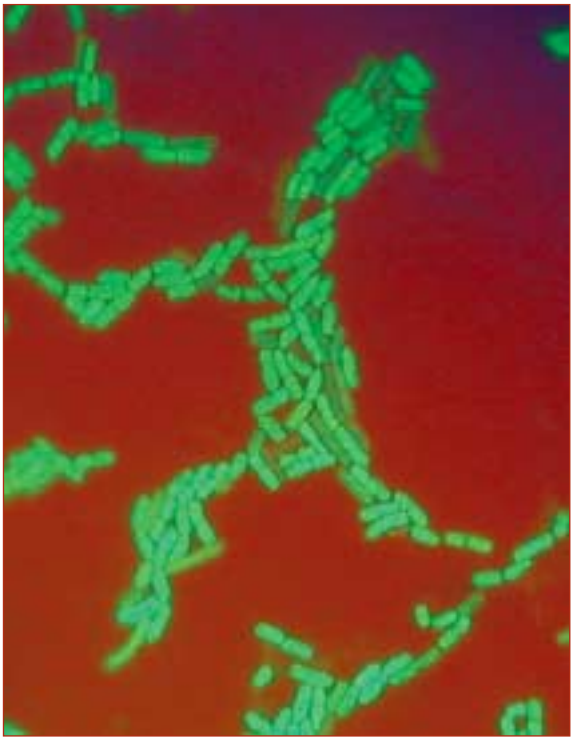

Figure 6.1. Fluorescently stained bacterial cells isolated from soil.

Microbial Biodiversity

To many people the term biodiversity usually means animals or plants; however, the soil habitat harbours a huge number of different microbial species - one gram of grassland soil contains about 10^3-10^4 different bacterial species. Whereas animal and plant ecologists can count numbers of different species through (relatively) easily identifiable traits, it is difficult to do this with bacteria and fungi. Until recently, microbial identification usually required isolation from soil through growth on standard laboratory media; however fewer than 1% of bacterial species and an unknown percentage of fungi could be recovered in this manner. Traditionally, i.e., pre-molecular biology days, the diversity of bacteria was determined firstly by culturing in the laboratory followed by a number of phenotypic tests; hence the true diversity of microorganisms in the soil remained unknown. This problem has now been circumvented through the application of molecular biological approaches in the nucleotide sequence analysis of ribosomal RNA genes (the 16S rRNA genes in bacteria and 18S rRNA genes in fungi). This approach avoids the problems associated with culturing because it utilises the total microbial community DNA extracted directly from soils. This DNA is used as a template for the polymerase chain reaction (PCR) amplification of ribosomal genes from all of the

members of that community. The individual gene products can be cloned and then sequenced to reveal the identity of the bacterial species from which the original target sequence was amplified. This approach, therefore, provides an insight into the identity of the remaining 99% or so of bacteria species, which cannot be readily recovered from soil by culturing techniques. In the past 12-15 years, since adopting molecular approaches, microbial ecologists have identified numerous new species of bacteria in soils, many of which are still only known by their 16S rRNA gene sequence alone, and many of these are now known to be numerically dominant in their respective habitats.

The importance of microbial diversity in soils is still not fully understood. It has been suggested that those soils harbouring a greater diversity of microorganisms are more likely to be resilient to stresses such as, for example, hydrocarbon (e.g., petroleum, diesel or oil spillage) or heavy metal contamination, or long term water-logging. Human land use and agricultural practices have been identified as the most important factors affecting biodiversity. We can put this into context when we consider that approximately 50% of the land area of the UK comprises agricultural grasslands, of which about 85% receives nitrogen (N)-fertiliser. Previous biodiversity studies have revealed that an increased management of grassland results in a decrease in plant and animal diversity, and also microbial diversity as determined through the use of broad-scale DNA approaches. More recent advances in molecular biology now allow us to assess the impact of land management practices on the community structure of specific groups of microorganisms in soils.

Studies at IGER are attempting to understand the relationships between grassland management regimes, microbial community structure and function, and also to determine the interactions between microorganisms and plant root exudates. Previous studies on the Rowden long-term experimental plots at IGER North Wyke had informed us that differences exist in the processing rates of specific nutrient transformations in soils under different management regimes. Further to these initial findings, more recent results now suggest that those soils from plots under different management regimes harbour microbial communities that are indeed different to each other. The microbial community structure was determined through the use of PCR-based techniques that allow for high-resolution analysis of the bacterial community structure. Using microbial community DNA extracted from soils as the template, similar size fragments of the bacterial 16S rRNA genes were PCR amplified. The analysis of this heterogeneous mix of PCR products from the different bacteria was then analysed by denaturing gradient gel electrophoresis (DGGE), which separates the DNA fragments based on their nucleotide content rather than size alone. The benefit of this approach is that a molecular fingerprint of the community structure is generated for each soil, such that each band in each lane of the gel theoretically represents a different bacterial species (Figure 6.2).

Figure 6.2. PCR-DGGE banding profiles of bacterial communities in four different soils.

These PCR-DGGE molecular fingerprints are complex and often difficult to interpret, and more meaningful information can be obtained through the

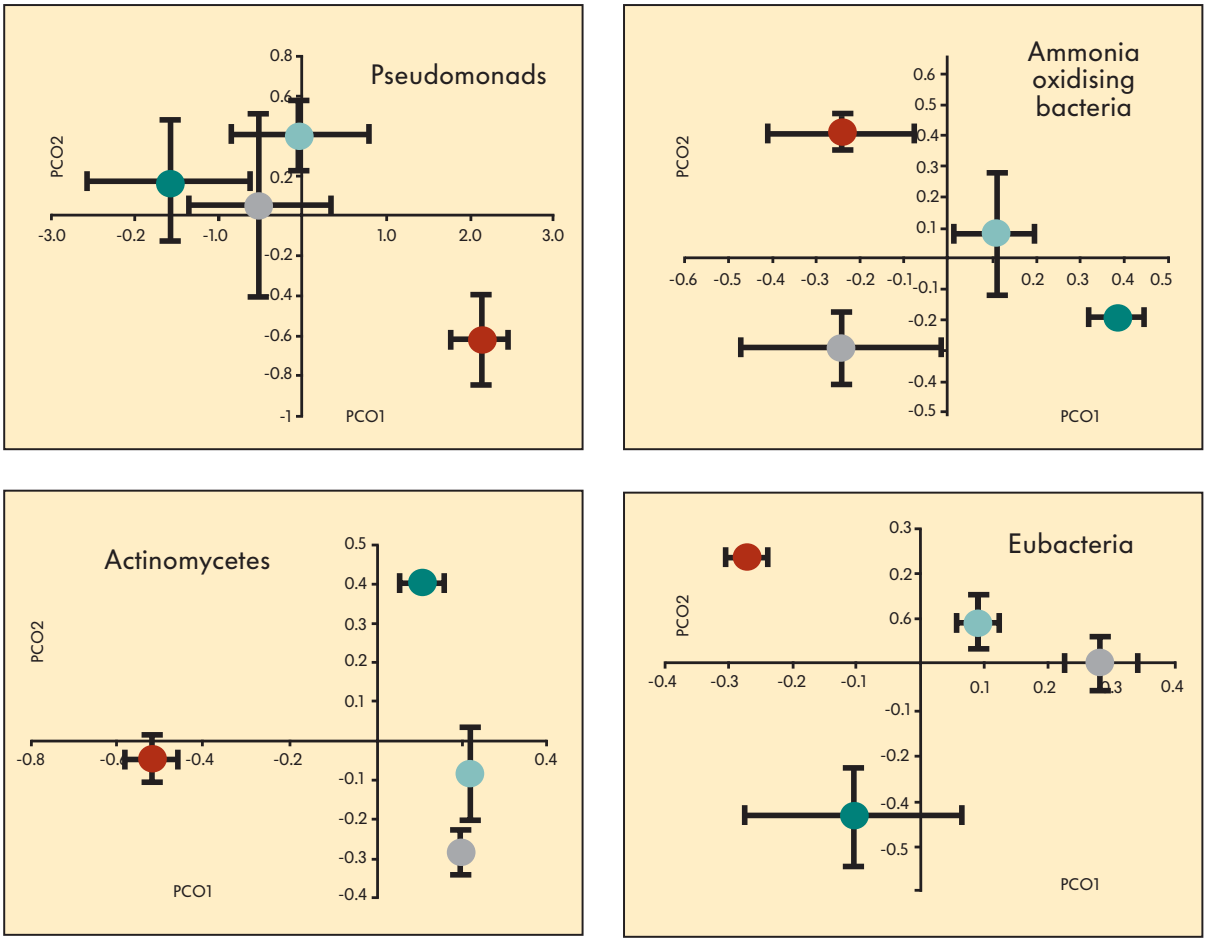

Figure 6.3. Principal co-ordinate plots of the community structure of the eubacteria, actinomycetes, ammonia oxidising bacteria and pseudomonads in soils from grasslands under different management regimes (unfertilised-undrained, unfertilised-drained, fertilised-undrained, fertilised-drained).

numerical analysis of the banding patterns. Population profiles have been generated for the general bacterial population (eubacteria), actinomycetes, ammonia-oxidising bacteria and the pseudomonads in plots under differing drainage and N-fertiliser status. The banding patterns obtained for these bacterial groupings have been transformed to produce the principal co-ordinate plots shown in Figure 6.3, and have revealed distinct clustering for each of the bacterial groupings where grassland management was the discriminating factor. These results provide us with some basic information regarding microbial diversity, and evidence that N-fertiliser and drainage are factors responsible for the development of distinct bacterial communities.

Plants can be instrumental in influencing the community structure and diversity of microorganisms in the rhizosphere soil through the release from the roots of soluble and easily diffusible compounds, (e.g., sugars, amino acids and organic acids) and also many insoluble compounds (e.g., cellulose, lignin and protein). Equally, soil microorganisms can influence which compounds the roots exude, thus creating a more favourable environment for themselves. One of the major factors that can influence root exudation is plant stress, whether by a gross effect, such as a defoliation event or insect attack, or a more insidious reduction in plant nutrient availability. These stresses

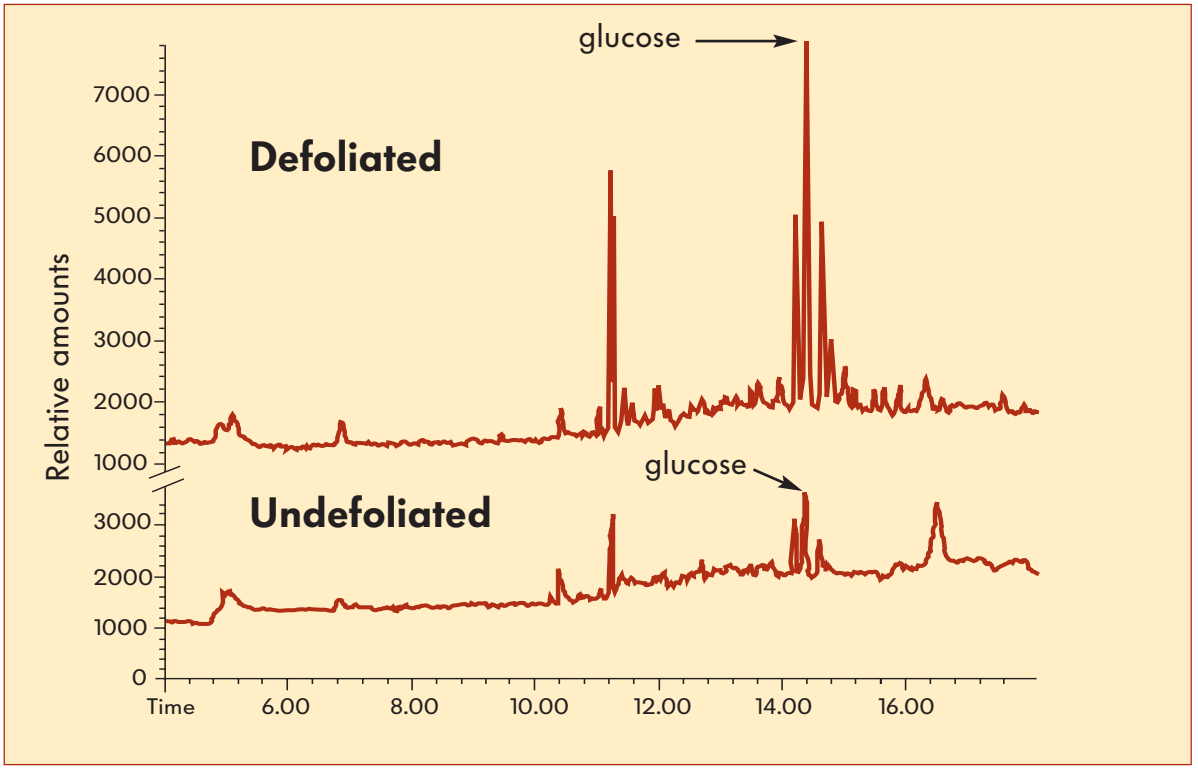

Figure 6.4. GC-MS separation of sugars in the root exudates of defoliated and non-defoliated Lolium perenne.

can radically alter both the quantity and quality of root exudates with consequences for the soil microbes.

For some detailed studies of microbial–plant–soil interactions it is necessary to work at a small scale. Current studies at IGER are determining the fine scale impacts of root exudates from *Lolium perenne* (perennial ryegrass) on rhizosphere bacterial populations of plants grown in rhizotrons consisting of a 2 mm thick layer of soil sandwiched between two glass plates. Fine scale sampling has revealed distinct spatial differences in bacterial population composition at different locations within the root zone. The work has also demonstrated the transient nature of bacterial community structure in the rhizosphere. Research undertaken using high performance liquid chromatography (HPLC) and gas chromatograph / mass spectroscopy (GC/MS) techniques has revealed that glucose is one of the dominant carbon compounds found in the root exudates of *Lolium perenne*, and that defoliation results in a change in both the quality and quantity of the carbohydrate profile (Figure 6.4).

Although much progress has been made in furthering our understanding of the relationships between plant roots and microorganisms, it is still unclear how microbial community structure and function are related in these soils. We are currently undertaking studies utilising stable isotopes to elucidate such relationships.

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