

While greenkeepers continue striving to maintain golf courses to the highest possible standard, research scientists are working in laboratories on projects that could ultimately eliminate some of the obstacles to perfect course conditioning. This month Dr Sue Grayston, Principal Scientific Officer in the Plant Ecophysiology & Rhizosphere Processes Programme at the Macaulay Institute in Aberdeen, gives an insight into some of the work that is being carried out at the Institute on soil biodiversity.

# Small but perfectly formed

Figure 1: 'A' Root



Figure 1: 'B' Root

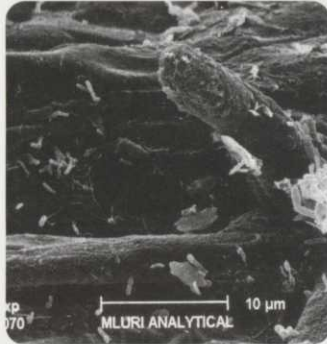


Figure 2

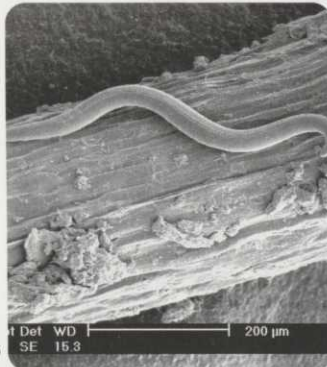
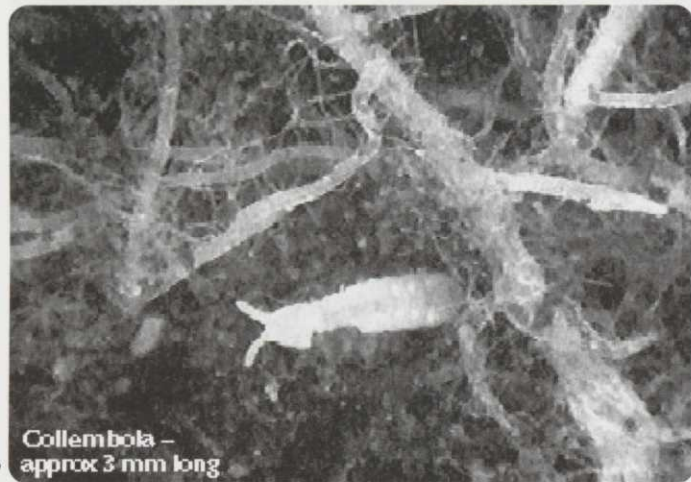


Figure 3



Did you know that soil probably harbours most of our planet's undiscovered biodiversity (Tiedje et al., 1999)? And yet we know very little about it. However, too small to be seen no longer means insignificant or valueless. Soil organisms are the key contributors to nutrient cycling, energy flow and storage in soil (Whitford, 1996). The soil biota recycle nutrients, produce and consume gases that affect global climate, destroy pollutants, treat wastes and can be used for biocontrol of plant and animal pests. Soil micro-organisms are the major sources of pharmaceuticals, antibiotics, immunosuppressants, enzymes and anti-tumour agents.

Soil organisms can be conveniently classified according to size. There are micro-organisms, which are invisible to the naked eye. They include bacteria, fungi and algae (Figure 1). These are the primary decomposers of plant and animal detritus in soil. The microfauna are the soil animals, less than 200µm long, therefore, also too small to be seen by eye and they include protozoa and nematodes (Figure 2). Mesofauna are animals of medium size (200µm - 1 cm in length) (collembola, mites, enchytraeids) (Figure 3) and macrofauna are animals who are cm's long, (earth-

worms, molluscs, arthropods, vertebrates) (Figure 4). Some soil fauna are predators of soil micro-organisms and other soil fauna, some are detritivores and others are plant herbivores.

Mature forest soils appear to have a diversity greater than any other habitat, with the possible exception of coral reefs (Behan-Pelletier & Bisset, 1992). The development and application of new methodologies to characterise, isolate and identify soil biota has indicated that we have only scratched the surface of soil biodiversity. Andre et al. (1994), using a new flotation method to extract mites from soil hypothesised that 10 million mesofauna in soil remained to be discovered. The situation is more dramatic with soil micro-organisms. Using molecular techniques it has been estimated that 1.5 million fungal species exist, yet only 5% are described. Similarly, for bacteria there may be 300,000 to 1 million species on earth, yet only 3,000 are described. A typical gram of soil contains 1 billion bacteria, only 10% of which are culturable (Torsvik, 1990a) and there may be 4,000 different microbial genomes present (Torsvik, 1990b). The total number of microbial cells on earth is far greater than individual animals and plants (Meyer, 1994). The survival of micro-organisms does not require plant or animal life, whereas the existence of micro-organisms is vital for plants and animals, including humans, and life on earth would cease if they became extinct. In fact, soil and its biotic component has been described as "our most precious non-renewable resource" (Marshall et al., 1982).

Bearing in mind the importance of soil organisms in ecosystem functioning and the fact that we know so little about individual identities and their specific roles it is vital if we are going to be able to manage different ecosystems effectively, or realise some of the potential untapped wealth waiting to be discovered in soil, we need more information on the char-

acter and functional significance of the soil biota. This statement holds true whether one is managing, at the one extreme a high input intensive grassland ecosystem, such as a golf course, or at the other extreme, a low input extensive pasture used as a grazing resource for animals. We also need to understand the key factors which affect the diversity and functioning of soil organisms. Availability of carbon (C) is a key factor affecting the growth of soil organisms. Therefore, any factor affecting soil C inputs will impact on the soil organisms. These factors include a) plant species - which vary in the amount and type of C they release to the soil, b) above-ground cutting, whether this be by an animal or a machine, - which alters C fluxes to the soil, c) below-ground root herbivory by insect larvae and nematodes - which increases C flow to the soil and d) soil amendments - which are used in intensive systems like golf courses as microbial stimulants.

Until recently it was felt that trying to identify the diversity of the whole soil microbial community was an impossible task because the techniques were just not available to study these small organisms. However, with the development of new molecular techniques like denaturing gradient gel electrophoresis (DGGE) to produce molecular fingerprints of microbial communities (Figure 5) and new chemotaxonomic techniques like phospholipid fatty acid (PLFA) profiling, which identifies characteristic 'signature' lipids found in the membranes of specific microbial groups, it is now possible to identify the total microbial diversity in soil. In addition, methods such as community level physiological profiling (CLPP) are being used to assess the functional diversity of the microbial communities in soil. The technique measures utilisation of a number of different carbon substrates (Figure 6) by microbial communities and is therefore relevant to soils, where microbial growth is carbon lim-

Figure 6

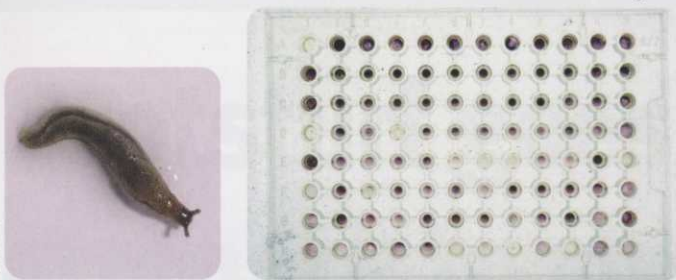


Figure 4



Figure 8: Bacteria



Figure 8: Fungi

ited. We have been undertaking this research at the Macaulay as part of these biodiversity programmes.

The use and further development of these techniques, applied to the research areas described above, is being undertaken at the Macaulay Institute in Aberdeen, as part of our core research programme funded by the Scottish Executive Environment and Rural Affairs Department (SEERAD) and in our role as partners in two major UK programmes on soil biodiversity – the SEERAD Micronet Project and the Natural Environmental Research Council (NERC) Soil Biodiversity Programme. Both of these programmes are centred on the grasslands at the Macaulay Institutes Research Station near Yetholm in the Scottish Borders. Although this research is being undertaken on upland grasslands the research and findings are equally relevant to intensively managed grasslands, such as golf courses.

The SEERAD Micronet Project (<http://www.scri.sari.ac.uk/MICRO>) is a major 10 year (1994-2004) co-ordinated programme on soil microbial diversity which is developing and applying a suite of molecular and phenotypic techniques to characterise the spatial and temporal diversity of soil microbial communities across a range of pastures differing in management intensity at 10 sites in the UK (Figure 7). The overall aim is to try to understand how soil microbial community structure affects the fertility of the soil and

the composition and productivity of the sward. This is a major programme of international significance; there is no other programme in the world applying a suite of both molecular and physiological techniques to the same soil samples to quantify spatial and temporal diversity of soil microbial communities.






We have been able to show that microbial community structure and activity is significantly influenced by grassland type and site, with intensively managed ryegrass, clover grasslands being a bacterial dominated system and the low input, extensive bent, fescue grasslands being fungal dominated (Grayston et al., 2001) (Figure 8). We have also shown that different grasses select for the microbial community in the soil surrounding its roots – 'the rhizosphere', through the variety of different carbon compounds they release from their roots (Grayston et al., 1998) (Figure 9).

The NERC Soil Biodiversity Programme (<http://mwnta.nmwa.ac.uk/soilbio>) is a five year programme (1999-2004), which aims to quantify the diversity of the whole soil biota in a bent fescue grassland in the Scottish borders, subject to a variety of management inputs (addition of nitrogen, lime, insecticide) (Figure 10). The key objective is to ascertain whether there is a link between biological diversity and function in soil. As part of this programme the Macaulay Institute, in collaboration with the Institute of Grassland and Environmental

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# Small but perfectly formed



Figure 13

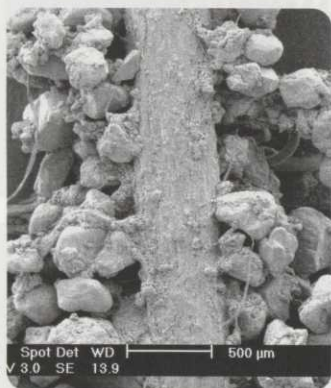


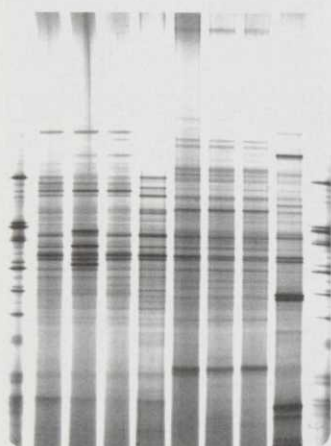
Figure 9



Figure 11



Figure 12



Gel



Figure 7

Research, North Wyke and Aberystwyth and Royal Holloway, University of London is quantifying the diversity of insect and nematode root feeders in grasslands and their impact on rhizosphere carbon flow and soil microbial communities.

This is the HUGBUG Project: (<http://www.mluri.sari.ac.uk/HUGBUG>)

Below-ground root herbivory by insects and phytophagous nematodes can result in qualitative and quantitative changes in soil carbon fluxes, which may impact on soil microbial diversity and functioning. We know something of the insect and nematode species that cause damage to agricultural grassland, and they are the same as those found in golf courses. However, the potential impact of root feeding by invertebrates on microbial driven nutrient cycling has not been evaluated.

Leatherjacket larvae (*Tipula paludosa*) are the dominant insect root herbivore in our bent fescue grassland (Figure 11). These larvae are also one of the biggest pests of turfgrass (see article by Yelland, Greenkeeper International, November 2000). In laboratory experiments at the Macaulay we have shown that root feeding by these larvae appears to be



Figure 10

plant species specific, the larvae significantly reduced the root biomass of ryegrass and clover, but not bentgrass. We have also shown, using miniaturised camera systems inserted into the soil, that these larvae differ in their root feeding patterns, preferring the laterals of ryegrass and the main roots of clover. Root herbivory by these larvae also resulted in increased C release to the rhizosphere beneath ryegrass and clover, which altered the microbial community structure in these soils. This change in microbial communities could obviously impact on soil functioning. Additionally,

the preferential feeding of this larva on different plant species could have an influence on plant community succession and explain why some plant species are better competitors. Currently, we are now assessing the impact of another insect root herbivore *Sitona* (Figure 12), and phytophagous (plant root feeding) nematodes on plant roots, rhizosphere C flow and the soil microbial community.

As part of our core funded research at the Macaulay we are assessing the impact of above-ground herbivory (frequency and height of cutting) on grass shoot and root growth, rhizosphere C flow, soil microbial communities and soil chemistry (Figure 13). We have been able to show that plants do differ in their response to cutting – e.g. bentgrass shows a significant reduction in root biomass with regular defoliation, ryegrass is more tolerant of cutting, in terms of shoot re-growth, than fescues, and bentgrasses, but not fescues can adapt their morphology in response to cutting, producing a shorter leaf growth zone. In addition, some plant species, like fescues lose more C from their roots into the soil after defoliation, which stimulates

growth of the soil microbial community.

All these responses can impact on the competitive ability of the different grasses, which we are trying to identify.

Microbes provide the underpinning of all ecosystems, therefore, it is vital we identify their diversity, function and the factors which affect their community size, structure and activity. By understanding how frequency and height of cutting, fertiliser, lime, pesticide application impact on the plant shoot and root growth, C fluxes and soil microbial communities and soil chemistry it will help us to understand why some plants are better competitors under different conditions and enable us to develop better management tools for our systems.

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The Macaulay's analytical division provide a wide range of QA soil chemical analyses. They also provide expertise on soil microbiological analysis, plant identification and analysis. They can also supply knowledge on the key processes governing plant growth and competition and welcome involvement in collaborative projects in these areas.

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