Biological Diversity and Ecosystem Function in Soil

Soil Biodiversity

NERC Thematic Programme

Hygrocybe laeta at Sourhope - Gareth Griffith

Newsletter - Issue number 9b

First issued March 2003
Reissued March 2004

Welcome to this 'bumper issue' of the Soil Biodiversity Newsletter. Early 2003 is certainly an auspicious time for those of us interested in soils. The NERC Thematic Programme is delivering many interesting and useful results; the British Ecological Society has used 'soil biodiversity' as the theme for its annual symposium; and there are stirrings in both the United Kingdom and the European Union about soil sustainability strategies.

The NERC Thematic Programme, with the official title 'Biological Diversity and Ecosystem Function in Soil' (usually shortened to 'Soil Biodiversity') started in 1997 with the establishment of a Steering Committee. There was plenty to do before a programme of co-ordinated, co-operative and integrated research began! The programme needed to be managed, a field site needed to be chosen and managed, an experimental design needed to be discussed, researchers needed to be funded (by the usual procedures of peer review, grading and selection to make a programme), and planning was needed for analysis, quality and archiving of data.

Soil Biodiversity site at Sourhope

The field site at Sourhope, managed by The Macaulay Institute, occupies about 1ha of acidic grassland. There is a randomised block design, with five replicates of six treatments. To be as close to agricultural practice as possible, the two main treatments are applications of nitrogen fertiliser and lime at normal rates, and a third treatment applies both nitrogen and lime. To explore perturbations, a fourth treatment applies a pesticide, Dursban 4. The experimental area is cut, with the cuttings removed, to simulate grazing (without the spatial effects of dung and urine deposition).

The research aims to understand the function of the biodiversity at this one site by a variety of experimental approaches. These vary from large scale experiments in the Ecotron (several tonnes of Sourhope soil were transported to Silwood Park) to very small studies on only a gram of soil.

The following articles, which cover the 30-or-so projects that make up the programme, are wide-ranging, from field survey to a variety of molecular methods, from the use of a $^{13}CO_2$ pulse to mathematical models, and involving the study of nearly all of the taxonomic groups. I suspect that it is true that we know more about the biodiversity of the Sourhope soil than any other soil on this planet.

I am privileged to chair the Steering Committee. As the Programme draws to an end over the next year or two, there are two important issues to address. First, how do we synthesise what has been achieved? The BES symposium will assist, but importantly the Programme is funding a group from the Universities of York and Cambridge to develop generic models of the soil ecosystem. Second, how do we put the research findings to the greatest possible use? This is a challenge that the Steering Committee still has to face; any ideas from readers of this Newsletter will be most welcome. I hope that, like me, you find much of interest in this Newsletter.

Michael B Usher

NATURAL ENVIRONMENT RESEARCH COUNCIL

More information about the Soil Biodiversity Programme, including details of the field site, full details of the project teams, published outputs and links to related sites can be found on the Programme's web site at the address below.

Website: http://soilbio.nerc.ac.uk

Bacterial diversity and activity in Sourhope soil by Rob Griffiths

Bacteria are primary decomposers of deposited organic material in soil, and therefore contribute to nutrient cycling vital to the functioning of the soil ecosystem. However, little is known of the factors driving bacterial community structure and functioning, and how resilient these communities are to change. We have used modern molecular, microbiological, and isotopic techniques to investigate how natural environmental changes may affect bacterial communities in Sourhope soil.

Initially, we aimed to determine the extent of temporal and spatial variation occurring in the Sourhope soil bacterial community. We discovered little spatial variation in replicate cores from a localised area, yet marked differences were apparent down the stratified soil profile. This was characterised by changes in diversity profiles based on DNA (indicative of the total diversity) and RNA (the "active" diversity), coupled with a decrease in culture-based activity with increasing depth. This variation is consistent with changes in physical and chemical gradients observed by other researchers in the program. There was also evidence of temporal shifts' with July samplings showing greater activity and diversity changes, possibly relating to increased plant activity and more favourable environmental conditions.

The influence of water stress upon bacterial communities was assessed in Sourhope turves by controlled water content manipulation (Figure 1). Here the objective was not only to examine changes occurring in diversity and activity as a result of drying and re-wetting, but also to identify functional groups of bacteria involved in carbon cycling. Bacterial activity decreased when soils were dried and recovered after several weeks of re-watering. However, no differences in total community diversity could be observed resulting from the imposed moisture regimes. Furthermore, no changes occurred in the total cell count, implying that water availability affects community activity without impacting on the total community per se. Changes in the 'active diversity' were however apparent after flow cytometric cell sorting and molecular analyses, indicating that minor differences in the active diversity are not sufficient to alter the observed diversity at the community level. This reinforces the idea that the total diversity pool in soil is large, which may confer a resistance mechanism to environmental perturbations.

Figure 1. Picture showing the impact of moisture limitation at the end of the drought experiment. Dried soils are clearly visible whereas re-watered pots appear indistinguishable from the continually wetted control treatments.

Finally, a $^{13}CO_2$ pulse labelling experiment was performed, in collaboration with colleagues at CEH Merlewood, to specifically target microbes utilising plant exudates. We aimed to assess whether RNA stable isotope probing (RNA-SIP) could be used in soil systems to identify organisms assimilating labelled rhizodeposits. Analysis of rhizosphere soil RNA (courtesy NERC Stable Isotope Facility) revealed that only the continually wetted treatments exhibited ^{13}C incorporation (Figure 2). However, enrichment levels were low and were insufficient for the application of RNA-SIP methods under this pulsing system. Interestingly, our data reveal that only a small percentage of fixed CO_2 is assimilated by the soil community from a single day's photosynthate.

As part of our Phase II project, and in collaboration with our co-workers, we are seeking new ways to more effectively target and identify these important functional communities.

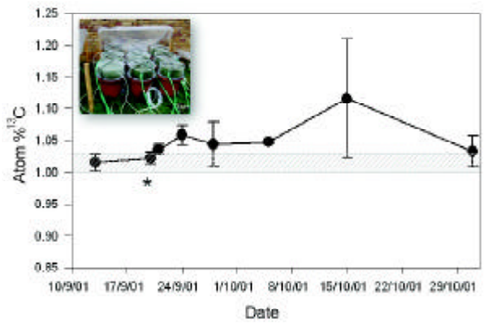

Figure 2. Incorporation of $^{13}CO_2$ into roots associated with continually wetted Sourhope soil. * indicates the date of $^{13}CO_2$ pulse labelling, and error bars represent standard deviation (n=3). Horizontal bar shows natural abundance values of soil RNA extracted prior to the pulse labelling. Inset: Delivering $^{13}CO_2$ pulse to Sourhope turves.

Examining relationships between microarthropod diversity, productivity and soil functioning by Lisa Cole

This project set out to test the applicability of current aspects of ecological theory developed for aboveground organisms, to a belowground community, the soil microarthropods. This group of soil animals comprises the mites (Acari) and springtails (Collembola) that together are the most numerically abundant and diverse non-aquatic faunal group in grassland soils. The study proceeded in two phases: (i) field-based experiments to identify patterns of diversity of microarthropods along gradients of physical disturbance and nutrient stress; (ii) a laboratory-based study that examined whether increased diversity of these soil animals was a driver of soil function, with respect to nutrient mineralisation and, therefore, plant growth and nutrition.

Our first objective, to identify patterns of microarthropod diversity along a gradient of increasing soil fertility and net primary productivity, was achieved through nutrient manipulations at the Sourhope site that produced a gradient of soil fertility and plant productivity. We found that whilst microarthropod densities and biomass increased concurrently with both soil fertility and net primary productivity, no change was detected in the diversity of soil microarthropods along the fertility gradient. However, communities of more fertile, productive sites contained a greater proportion of predators, and the diversity of one group, the collembola, was marginally enhanced at more productive sites.

We also explored additional experimental manipulations that imposed severe physical disturbance as well as stress, in the form of nutrient availability, on this grassland system. This study revealed that disturbance reduced microarthropod community size and diversity, and was a more important determinant of the structure and biomass of the microarthropod community than was resource availability.

Our findings suggest, therefore, that unlike in plant communities, soil faunal diversity is not strongly regulated by competition, and that competitive exclusion does not occur when soil resource availability is increased and soil conditions are improved. In addition, no relationship was found between measures of plant and animal diversity at the scale of our individual sampling unit, the soil core, suggesting that other factors determine microarthropod community composition and diversity, such as variations in habitat complexity, which enables the co-existence of species through the avoidance of competition. The finding that physical disturbance of the soil had a strong, negative influence on the abundance and species richness of microarthropods supports this idea. The second, laboratory-based phase of our work involved the creation of microcosms containing model communities of microarthropods of increasing diversity (from zero to eight species mixtures) planted with seedlings of *Agrostis capillaris*, to determine whether increased soil animal diversity is a driver of soil function. We used a stable isotope labelled amino acid (glycine) as a pulse of organic nitrogen, traceable as ^{15}N in the soil, microbial, and plant pools. The handful of ^{15}N-tracer experiments that have been done on agricultural plants suggest that microbes compete extremely effectively with plants for organic nitrogen inputs, often sequestering most, if not all, of the ^{15}N that is added to soil (Hodge *et al* 1998, 1999; Bardgett *et al*. 2003). Our measure of soil function was the ability of microarthropods to influence the process of nitrogen acquisition by *A. capillaris*, through their interactions with the soil microbial community. We hypothesised that microarthropods would liberate ^{15}N that was immobilised from the glycine pulse by the microbes, enabling plants to acquire more ^{15}N from the pulsed nitrogen source. This would shift the balance of the competition for nitrogen in these soils from the microbes to the plants, resulting in a greater equilibrium of nitrogen derived from the glycine in the plant and microbial pools. Second, we hypothesised that this liberation of microbial ^{15}N would be greater in a more diverse community of microarthropods.

We found that whilst soil microarthropods had no effect on plant productivity, two individual collembolan species *Protaphorura armata* and *Pseudosinella alba*, were effective in reducing microbial sequestration of added ^{15}N. For one species (*P.alba*) this translated into increased capture of ^{15}N by *A.capillaris.* Despite these single species effects on nitrogen cycling in soil, we found that increasing soil animal diversity had no beneficial influence on ^{15}N capture by the plants, and therefore, plant nutrition and the potential productivity of the system.

We conclude that the identity of soil microarthropod species in a community, rather than community complexity itself, is a more precise determinant of soil function, and that the effect of soil animal diversity on soil function is idiosyncratic, being dependant on the species identities within the community and the consequence of their biotic interactions within and between soil communities.

Hodge, A. Stewart, J. Robinson, D. Griffiths, B.S. Fitter, A.H. (1998). Root proliferation, soil fauna and plant nitrogen capture from nutrient-rich patches in soil. New Phytologist 139: 479-494.

Hodge, A. Stewart, J. Robinson, D. Griffiths, B.S. Fitter, A.H. (1999). Plant, soil fauna and microbial responses to N-rich organic patches of contrasting temporal availability. Soil Biology & Biochemistry 31: 1517-1530.

Diversity and function of enchytraeid worms in a Scottish upland grassland: a dual molecular approach by Helaina Black

Only recently has the role of enchytraeids (Figure 3) in soil carbon cycling in natural and semi-natural ecosystems been realised. Despite this, the importance of individual enchytraeid species or group diversity in maintaining carbon cycling remains largely unknown. Although enchytraeids are generally classed as omnitrophic, there is some indication that species may have different feeding preferences.

These differences may in turn affect the overall contribution of the enchytreaid community to carbon cycling since we already know that agricultural practices and pollution can alter community structure.

Our lack of understanding of the diversity and functional activity of enchytraeid species is largely due to taxonomic and laboratory difficulties. To address these, we integrated existing taxonomic expertise with molecular genetic techniques to produce a robust phylogeny and durable set of genetic markers for enchytraeid species at Sourhope. We then used genetic markers and stable isotope analysis to identify and measure carbon uptake by individual worms sampled from the field, after a pulse of CO_2 enriched CO_2. We used this approach to examine the impact of a liming induced change in enchytraeid community structure and, especially, a reduction in a pre-dominant species (*Cognettia sphagnetorum)*, would have on carbon cycling by enchytraeids.

Enchytraeid-specific polymerase chain reaction primers were generated for each worm identified to genus.. In most cases, each putative enchytraeid genus, identified using classical approaches, represents a monophyletic clade. The one exception is *Marionina argentea,* which falls into the *Fridericia* clade. Sequence variation at the nuclear 18s rDNA gene was utilised to underpin a high-throughput species identification procedure. This permitted identification to species level and characterisation of community structure.

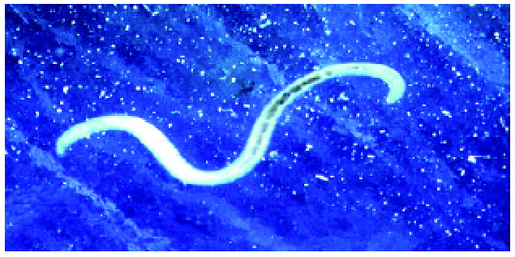

Figure 3. An enchytraeid from Sourhope

Fourteen species from four genera (*Cognettia, Fridericia, Henlea* and *Achaeta*) were recorded from Sourhope. *Cognettia* was the most common group in unimproved pasture and was dominated, as expected, by one species, *Cognettia sphagnetorum*. Management by liming reduced enchytraeid abundance, but not biomass, by reducing *Cognettia* spp. numbers (especially *C. sphagnetorum*) while species richness increased, especially in *Fridericia*). Stable isotope analyses of cholesterol from each worm sampled after the *in-situ* isotopic pulse demonstrated that liming reduced rhizosphere carbon assimilation by enchytraeids.

This overall reduction was the product of a change in carbon assimilation by the two dominant genera, *Cognettia* and *Fridericia*. The reasons for differences in carbon assimilation between these are, as yet, unclear but may relate to microbial and/or organic matter preferences. Our results do indicate that enchytraeids assimilate rhizosphere carbon relatively quickly and suggest that relative contributions of major plant sources (root versus litter) should be assessed to establish whether an increase in enchytraeid diversity, which can be brought about by management, would result in a reduction in the contribution of enchytraeids to soil carbon cycling.

These results, although from one site in one year, have important implications for how we quantify the role of enchytraeids in soil carbon cycling. Accurate estimates and predictions of the extent to which soil fauna contribute to soil carbon cycling are needed in order to improve large-scale carbon models, which are used to address the sustainable use of soils and other environmental concerns such as climate change.

Interactions of soil biodiversity, micromorphology, structure and organic matter
by Patricia Bruneau

Our project investigated how soil faunal activity and community composition influences soil structure and soil properties at Sourhope. Emphasis was given to comparing results from the limed and control plots. Use was made of a combination of approaches, for example, quantitative soil micromorphology, aggregate stability and tracing the movement of ^{13}C into the soil fabric. Similar approaches were also used to investigate the impact of faunal communities and nitrogen amendments on soil analogues created in the Ecotron microcosm at Imperial College.

An initial soil survey of Sourhope was carried out to reveal differences in drainage condition and sequences of upper horizons. Three upper horizon sequences were identified that had developed after abandonment of cultivation c1750. Davidson (2002) discusses the effect of old cultivation on soil properties and concludes that the effects of bioturbation are dominant. Davidson, et al (2002) discuss the nature of the upper horizons prior to treatment.

Although there is considerable biodiversity in the soil, around 90% of the organic matter has been processed by two major animal groups, enchytraeids and earthworms. There is a close association between the nature of the upper horizons and excremental features from these groups, for example the distinctive dominance of enchytraeid excrement in the Ah horizon. Over the 2 year experimental period we saw increases in earthworm excrement in particular soil horizons in the control plots and a corresponding decrease in undifferentiated (aged) excrement. The effect of liming was also to increase earthworm excrement and decrease the amount of undifferentiated excrement.

Image analysis techniques were developed to isolate large excremental features associated with earthworms and enchytraeids. Davidson, Bruneau and Grieve (submitted) demonstrate the relationships between change in soil structure as expressed by differences in void space and excremental features. Furthermore, using a specific fluorescent dye, the spatial distribution of soil bacteria in soil thin sections and their relationship with excremental features was investigated. Bacterial counts in limed plots were higher in both the H and Ah horizons. This is related to a higher density of bacteria in the H horizon associated with enchytraeid excrements whereas in the Ah horizon the higher density is linked to earthworm excrements.

Bruneau, et al (2002) developed an innovative technique laser ablation stable isotope ratio mass spectrometry, LA-IRMS that provided spatially distributed data on a pulse-derived ^{13}C tracer from roots. ^{13}C enrichment in roots was shown to be very variable even within single roots. No significant enrichment in soil excremental features was seen over the duration of the experiment. The variability of the ^{13}C signal in root material was demonstrated. The method has since been applied successfully in field conditions in phase II of the programme.

Changes have also been found in aggregate stability as a result of liming, in part related to increases in earthworm activity. The experimental results from the field and microcosm were compared and show similar trends in relationships between soil organisation and impact of disturbance.

Overall, our project project has highlighted the complex relationships between soil organisation and soil faunal communities. Most of the soil carbon in soils at Sourhope occurs in excremental features of either earthworm or enchytraeid origin. These species play key roles as soil bioturbators, but also provide more easily assimilated carbon to the rest of the soil ecosystem.

Bruneau, P.M.C. Ostle , N. Davidson, D.A. Grieve, I.C. Fallick A. (2002). Determination of rhizosphere ^{13}C pulse signals in soil thin sections by Laser Ablation Isotope Ratio Mass Spectromery (LA-IRMS). Rapid Communication in Mass Spectrometry 16: 2190-2194.

Davidson, D.A. (2002). Bioturbation in old arable soils: quantitative evidence from soil micromorphology. Journal of Archaeological Science, 29: 1247-1253.

Davidson, D.A., Bruneau, P.M.C. Grieve, I.C. (submitted) An evaluation of image analysis for measuring changes in void space and excremental features on soil thin sections in an upland grassland soil. Geoderma.

Davidson, D.A. Bruneau, P.M.C. Grieve, I.C. Young, I.M. (2002). Impacts of fauna on an upland grassland soil as determined by quantitative

Biodiversity of invertebrate root herbivores and their impact on soil microbial communities by Lorna Dawson

We surveyed insect populations in the soil at Sourhope at regular intervals. In 1999 *Tipula paludosa*, was the dominant insect root feeder with populations reaching 250 per m^2 *Scarabaeidae* (Chafers), *Noctuidae* (Cutworms), *Bibionidae*, *Curculionidae* and *Elytreideae* were also present, but at relatively low numbers. Numbers of Tipulid larvae decreased with time, whereas the numbers of cutworms increased over the study period.

Figure 4. Sample collection in the field at Sourhope

This may be due to changes in the sward structure with the change from a lax sheep grazing system, producing relatively tall coarse grassland, to an intensive cutting regime that resulted in a shorter sward. All insect numbers were reduced by insecticide treatment. Nematode numbers (averaging 4.5 x106 per m^2) were reduced by 43% in re-seeded sub plots compared to the control swards, averaged over treatments and sampling dates. In both swards (i.e. natural and reseeded with *Lolium perenne)* swards there were 35% fewer nematodes in plots treated with nitrogen and lime, but this response was seen earlier and to a greater extent in the re-seed treated sub plots.

Different trophic groups of nematodes were affected in contrasting ways by the treatments: nematodes associated with root hairs or fungal hyphae (many tylenchid taxa) were smaller proportions of the population in reseeds where there were more bacterial feeders than in natural sward.

In N +L plots there were more bacterial feeders but fewer plant-hyphal and fungal feeders. Predatory nematodes became more prominent in N+L treated reseeded sub-plots in 2000 and 2001. Dominant taxa of nematodes responded differently to the major influences of reseeding and treatment with N+L: other main treatments had little effect. Of the three principal root feeding taxa, *Helicotylenchus* sp. increased with time in the reseeds but was present in reduced numbers in the N+L plots; Paratylenchus declined markedly in reseeds and failed to recover; and it and Paratylenchus were unaffected by main plot treatments.

Twelve laboratory studies (Figure 5) were carried out to determine feeding effects of Tipulids, nematodes and clover weevils (*Sitona* spp.). In these studies, feeding by Tipulid larvae negatively affected the biomass of *Lolium perenne* and *Trifolium repens*, which are sub-dominants at the field site. The dominant grass species, *Agrostis capillaris*, was not affected.

Resistance to root feeding could play a role in the dominance of this grass. In addition, when exposed to roots of *T .repens*, Tipulid larvae fed voraciously on the main root axes, in contrast to a lesser effect on the root system of *L. perenne*. Larval feeding resulted in both qualitative and quantitative changes in rhizosphere solutions collected from these plants and changes were plant-species specific. In the field, root numbers were significantly less where insecticide had removed the root herbivore. In addition, the application of the nitrogen and lime reduced root longevity and increased carbon and nitrogen return to the soil. There was a strong seasonal trend in root appearance, with most new roots being produced in spring and early summer.

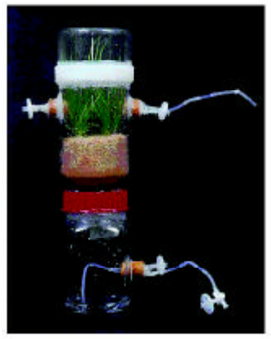

Figure 5. Microcosm system showing collection system for root exudates.

The treatment effects were similar in the re-seeded sub-plots, although root density was overall lower. In laboratory studies the impact of root herbivory on microbial diversity and activity was determined using community level physiological profiling (CLPP, Biolog) and Phospholipid fatty acid (PLFA) signatures. Root feeding significantly increased the amount of carbon flow to the rhizosphere in both *Lolium perenne* and *Trifolium repens* but not in *Agrostis capillaris*.
Root feeding increased the Pseudomonad community in the rhizosphere and changed the carbon utilisation profiles, indicating changes in bacterial community structure. Interactions between many trophic levels, from vegetation influences to those of the microbial community have been investigated in this study.

Nematode Barcoding: Identification and ecology through sequence
by Robin Floyd

Nematodes are abundant members of soil biotas worldwide. Due to their small size, their identification through morphology is properly the task of well-trained experts, and is both time consuming and difficult. We have been using the site at Sourhope to investigate new ways of performing identification of soil nematodes. Our hope is that these new methods will find utility in other soil-focussed nematode inventory/survey projects, as well as be applicable to other nematode biota, such as those of the marine benthos.

Our approach was to determine for each specimen the sequence of a fragment of the ribosomal small subunit RNA (SSUrRNA) gene, a "molecular barcode", and then use this sequence to define molecular operational taxonomic units (MOTU) and classify specimens to MOTU).

We chose the SSUrRNA gene because (1) it is relatively easy to isolate through PCR amplification, (2) it has both highly conserved and divergent areas making it suitable for both low-level ("species") and higher-level (genera to families) identifications, (3) there was already a database of sequences available from nematodes and (4) we reasoned that the data would be amenable to detailed phylogenetic analysis for the identification of novel specimens. In pilot projects we developed a rapid throughput, single-nematode DNA extraction method, and also determined the level of experimental error in sequence derivation. This error rate puts a lower limit on our discrimination between MOTU. Within a single specimen, or within different individuals of a single isolate, we find less than 2 base pairs difference between repeat sequencing of a 450 base pair region. Thus our heuristic is to define a MOTU as any specimens whose sequences differ by up to 2 base pairs in 450. To automate the MOTU-definition process, we have implemented genomics-style base-calling and analysis software, and have processed around 2000 specimens. These have been found to contain 140 different (2 base pair-) MOTU.

How do these MOTU compare to traditional taxa? In a series of cultured panagrolaim isolates we have determined MOTU, morphological taxa and biological species. The biological species division (i.e. interbreeding tests) of the isolates agrees with the MOTU designation, whereas the morphological system was unable to separate two of the taxa. We thus suggest that MOTU are at least as good as morphological taxa in defining real biological units (A. Eyualem and M. Blaxter, submitted).

Can we link MOTU to traditional taxa? The specimen preparation method we use results in the destruction of the specimen. Adding a digital photography voucher image step to the process resulted in an unacceptable increase in time taken per specimen, but directed sequence acquisition from selected specimens allowed us to attach species names to several MOTU, particularly for those we could maintain in culture. In addition, comparison of the barcode sequence to a database of sequences determined by ourselves and others from identified nematode specimens allows us to allocate putative taxonomic identifications to the MOTU. For MOTU whose sequences do not match a previously sequenced taxon within our heuristic (2 bp in 450) we are able to place the MOTU within a known higher taxon (genus, family). In this way we can add "biology" to our sequences.

The trials at Sourhope have shown that the method is feasible and efficient, and yields data comparable to other survey methods. Importantly, the raw data can be archived (for example in GenBank) and accessed at any time by others wishing to compare their surveys with ours, or any other molecular barcoding program. We are now investigating methods to increase efficiency and throughput, and applying it to marine and other nematode faunas (see http://www.nematodes.org/).

Blaxter, M.L. (2003). Molecular systematics: Counting angels with DNA" Nature 421: 122-124).

Floyd, R.M. Abebe, E. Papert, A. Blaxter, M.L. (2002). Molecular barcodes for soil nematode identification. Molecular Ecology, 11: 839-850.

Soil Protozoan Diversity and its role in carbon and nitrogen turnover
by Bland Finlay

Protozoa are an incredibly diverse group of organisms, both in terms of numbers and biomass. They occur in a wide range of habitats, including soil. This project looked at protozoan diversity and function at Sourhope, and also involved some problems in proozoan taxonomy.

We developed a method for determining the potential abundance of free-living protozoa in soil. The method permits simultaneous enumeration of the four major functional groups (flagellates, naked amoebae, testate amoebae, ciliates), and it can be used to compare the effects of different soil treatments (e.g. liming, biocides) on the soil protozoan community.

365 protozoan species were recorded from the Sourhope site. This represents approximately one third of the known global diversity of soil protozoan species. Such a high local:global species ratio is consistent with the developing consensus that microbial eukaryote species have cosmopolitan distribution.

With the soil protozoa, as in all other animal groups, abundance is inversely related to cell size. Median abundance in 150 soil samples collected at Sourhope ranged from 2000 ciliates (the largest species) per gram dry weight of soil, to 43000 flagellates (the smallest species) per gram dry weight.

Across the entire size range of soil protozoa, species richness varied by a factor of two whereas abundance increased by a factor of 20, with decreasing body size. The soil had fractal structure (Figure 6) so the likely explanation is that self-similar topography at progressively finer spatial scales supported constant species richness but increasing habitat area, and therefore increasing numbers of protozoa. A simple model based on the fractal dimension of soil and the weight-specific energy requirements of protozoa provided a good fit to the abundance – body size distribution when the protozoan community reached something resembling a steady state.

With respect to the natural history of individual protozoan taxa, we have improved our knowledge of poorly known taxa, especially the cercomonad flagellates, which are among the most abundant in Sourhope soils, and the thaumatomonads. We have resolved significant problems in the taxonomy of soil protozoa (e.g. *Gerda glans*; morphogenesis of *Uroleptus lepisma*), and we have re-described the ciliate *Psilotricha acuminata* as a 'missing link' between the Euplotidae and the Oxytrichidae. We have also produced a 'Video-guide to the identification *in vivo* of soil ciliate species'. We isolated and cultivated the predominant flagellates in Sourhope soils, investigated their life histories *in vitro*, and sorted out differences. We have isolated and investigated a novel voracious helioflagellate predator on other soil flagellates, and its method of feeding, and we have isolated and cultivated an apparently novel class of bacterivorous plasmodial organisms . These lack evident cytoplasmic flow, and are relatively common in Sourhope soils.

In microcosm experiments, soil protozoa were shown to stimulate the rate of carbon turnover – probably through their grazing activities.

The level of stimulation increased with increasing biological diversity in the treatment sequence: Bacteria; Bacteria + Amoebae; Bacteria + Ciliates; Bacteria + Amoebae + Ciliates. The rate of carbon turnover in the last treatment was 50% higher than that in the treatment with bacteria alone.

Using soil ciliates and testate amoebae, we have tested the hypothesis of ubiquitous dispersal of individual species. We compared data from 150 soil samples at Sourhope, and from 1500 soil samples collected worldwide. A fundamental pattern of random spatial distribution was found at Sourhope, and random dispersal appears to extend also to the global scale. Species that are rare or abundant locally, are likewise rare or abundant at the global scale.

It is likely that ubiquitous dispersal of microbial species is driven by their high absolute abundance, and this project has provided the first good data for the scale of that abundance. The median abundance of a soil protozoan species at the one-hectare Sourhope site ranges from 0.5 billion (ciliates) to 14 billion (flagellates) individuals.

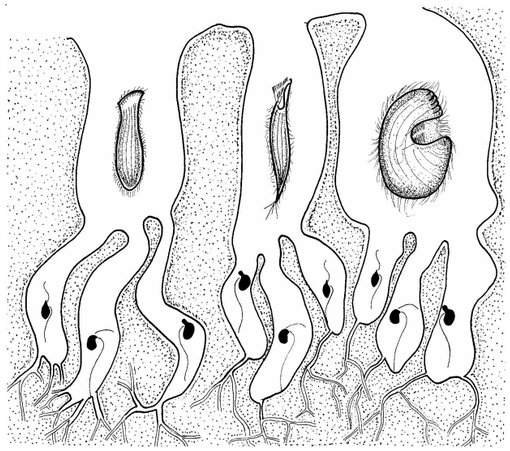

Figure 6 Schematic representation of protozoa in a fractal soil environment. The three larger protozoa are all ciliates and there are three species – each represented by one individual. The smaller protozoa are flagellates. Their habitat space is bigger, and they are more abundant than ciliates, but their habitat is qualitatively similar to that of ciliates, so there are still only three species. The smallest spaces and fissures are too small to be accessible to protozoa.

Finlay, B.J. Black, H.I.J. Brown, C. Clarke, K.J. Esteban, G.F. Hindle, R.M. Olmo, J.L. Rollet, A. Vickerman, K. (2000). Estimating the growth potential of the soil protozoan community. Protist, 151: 69-80.

Finlay, B.J. Esteban, G.F. Clarke, K.J. Olmo, J.L. (2001). Biodiversity of terrestrial protozoa appears homogeneous across local and global scales. Protist, 152 : 355-366

Vickerman, K. Le Ray D. Hoef-Emden, K. De Jonckheere, J. (2002). The soil flagellate Proleptomonas faecicola: cell organisation and phylogeny suggest that the only described free-living trypanosomatid should not be assigned to the Kinetoplastida. Protist 153: 9-24.

Finlay, B.J. (2002). Global dispersal of free-living microbial eukaryote species. Science 296: 1061-1063.

Olmo, J.L. (2000). Morphology and morphogenesis of *Uroleptus lepisma (Ciliophora, Hypotrichida*). European Journal of Protistology, 36: 379-386.

Esteban, G.F., Finlay, B.J., Charubhun, N. Charubhun, B. (2001). On the geographic distribution of Loxodes rex (Protozoa, Ciliophora) and other alleged endemic species of ciliates. Journal of Zoology, 255: 139-143.

Finlay, B.J. & Esteban, G.F. (2001). Exploring Leeuwenhoek's legacy; the abundance and diversity of protozoa. International Microbiology, 4: 110-116.

Finlay, B.J. & Fenchel, T. (2001). Protozoan community structure in a fractal soil environment. Protist, 152: 203-218.

Esteban, G.F., Olmo, J.L & Finlay, B.J. (2001). Re-discovery of a "missing link" ciliated protozoon. Limnetica, 20: 15-21.

Finlay, B.J. & Esteban, G.F. (2001). Ubiquitous microbes and ecosystem function. Limnetica, 20: 161-173.

Esteban, G.F. & Finlay, B.J. (2002). Historical encounters with a little-known ciliate (Gerda glans Claparède and Lachmann,1858) from the 'Jungfernheide'. Protist, 153: 79-86

Vickerman, K. Le Ray, D. Hoef-Emden, K. De Jonckheere, J.(2002). The soil flagellate Proleptomonas faecicola: cell organisation and phylogeny suggest that the only described free-living trypanosomatid should not be assigned to the Kinetoplastida. Protist 153: 9-24.

Influence of land use management practices on diversity, biomass and functioning of soil microbial communities and their role in carbon and nutrient cycles by Jim Prosser

Efficient functioning of soil ecosystems relies on the biogeochemical cycling of nitrogen. The major inorganic pools of nitrogen, ammonia and nitrate, are determined by two key processes within the soil nitrogen cycle: nitrification, the sequential oxidation of ammonia to nitrite and nitrate, and denitrification, the reduction of nitrate to gaseous products. These processes control nitrogen supply to plants, losses of gaseous nitrogen, leaching of nitrate and generation of greenhouse gases. This project focuses on the organisms responsible for the second stage of nitrification, the nitrite oxidising bacteria. These organisms convert nitrite to nitrate under aerobic conditions, but under anaerobic conditions can reverse the process, reducing nitrate.

Little is known of their diversity, so we used molecular techniques to study it. We also use microcosm and field studies to determine the influence of soil management processes on their community structure and on the rates of processes in which they were involved.

In microcosm studies soil moisture strongly influenced emissions of nitric oxide (NO) and nitrous oxide.(NO)emissions peaked under the driest soil conditions, whereas N_2O peaked in the wettest soils. Long-term enrichment of Sourhope soil showed that lime additions,in combination with other treatments, increased the emission of NO relative to N_2O (see Figure 7) and also increased molecular diversity. The contribution of denitrification to the N_2O flux was largest in the lime-treated soils; however in studies with ^{15}N complete **denitrification to N_2** was not detected. The distribution of ^{15}N in the N_2O molecules suggested that more than one process was responsible for N_2O production.

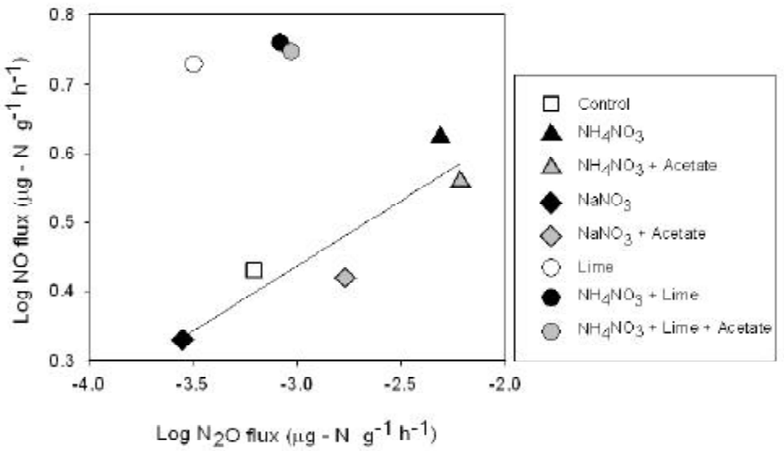

Figure 7. The influence of long term enrichment of NH_4NO_3 lime acetate and $NaNO_3$ on the emissions of NO and N_2O . The regression line was fitted to the non-lime treated soils only (r^2+0.75).

Bacterial communities were characterised by analysis of 16S rRNA genes. We found differences between eubacterial communities in microcosms treated with lime, nitrogen and organic carbon. Nitrite oxidiser communities were dominated by *Nitrobacter*-like sequences, with some evidence of increases in some sequence types following nitrogen amendment. Ammonia oxidisers however were dominated by *Nitrosospira* 40KI-like sequences, with *Nitrosospira* species AF-sequences increasing following nitrite amendment. Analysis of 16S rRNA genes from enrichment cultures of nitrite oxidisers indicated similarities with those amplified directly from soil but only one was identical to a sequence from a laboratory culture.In the **field** emission's of N_2O and NO and soil mineral nitrogen concentrations increased after nitrogen fertilisation with ammonium nitrate, with further increases in emissions in nitrogen and lime treated soils. Treatment effects were short lived and after 8 weeks gaseous loss of NO and N_2O was close to that of the control plots. Molecular analysis of ammonia oxidiser and *Nitrobacter* communities in field plots Indicated a reduction in heterogeneity following soil amendment but little variation in *Nitrobacter* profiles between plots.

In conclusion our study has helped us to understand the effects of some soil management practices on the diversity and functioning of a key group of soil organisms.

Flow paths and rates of labelled carbon transfer within the spatial organisation of an upland grassland soil by Ian Grieve

Monitoring of carbon fluxes by bulk soils analysis cannot provide sufficient detail on carbon fluxes within the soil structure, which is vital to the understanding of soil carbon pathways. The objective of this project was to investigate variations in transfers of labelled carbon between components of the soil structure with changing faunal biodiversity. ^{13}C was added to soils in the control, nitrogen limed and biocide plots in two contrasting ways, as a pulse of $^{13}CO_2$ (June 2002) and as ^{13}C enriched litter (October 2001).

Thin sections were made from undisturbed samples collected from the pulsed soils 0, 2, 7 and 35 days after the pulse and from the litter-amended soils. Laser-ablation isotope ratio mass spectrometry (LA-IRMS) was used to analyse the isotopic composition of soil features including roots, specific excrement features and adjacent soil. Full details of the methodology used in this work are in Bruneau *et al* (2002). Thin sections were also analysed by Electron Microprobe at Manchester University. Elemental maps of 5 elements (Fe, Ca, C, Mg, N) were produced for each section.

We made 270 successful measurements from the soil thin sections. Thin sections of soils at natural abundance were first analysed to determine mean ^{13}C for root and excremental features. This enabled us to determine threshold values for enrichment for both root & excremental features.

Enrichment varied with treatment, date of sampling and soil feature. Overall, less enrichment was found in the limed plots than in the control and biocide plots. There was also a significant difference in the mean ^{13}C of enriched samples. The limed plots show the lowest overall enrichment and soil organic features were very close to natural abundance. The biocide treatment had the highest enrichment values, particularly in roots .
When all the data are fully analysed we will have helped to understand some of the pathways of carbon flow in soils, and the importance of soil fauna in these pathways.

Bruneau, P.M.C. Ostle , N. Davidson, D.A. Grieve, I.C. Fallick A. (2002). **Determination of rhizosphere** ^{13}C pulse signals in soil thin sections by Laser Ablation Isotope Ratio Mass Spectromery (LA-IRMS). Rapid Communication in Mass Spectrometry 16: 2190-2194.

Diversity and functional role of predatory beetles and their prey in the Sourhope ecosystem
by Michelle Fountain

This project is investigating the functional role and diversity of specific groups of predatory beetles in the Sourhope soil and linking the above- and below-ground processes mediated by these groups.

We aim to demonstrate 'top-down' effects of predators in the Sourhope food web. Two scenarios may be envisaged. Firstly, reducing predator numbers will increase herbivore populations and enhance herbivory, resulting in reduced plant productivity. Alternatively, there will be increased microbivore abundance, through herbivore-mediated processes, leading to an increase in decomposition rates and plant productivity. To test this, invertebrate numbers have been assessed in selected treatment plots and predator/herbivore numbers will be manipulated in controlled experiments involving the removal of soil cores from the site.

Work began in July 2002 and species identifications are well underway (quite a task!). An attempt was made to estimate populations of the above- and below-ground life stages of carabids (significant predators of herbivores). However, from the 340 cores removed from the site in the spring and autumn,

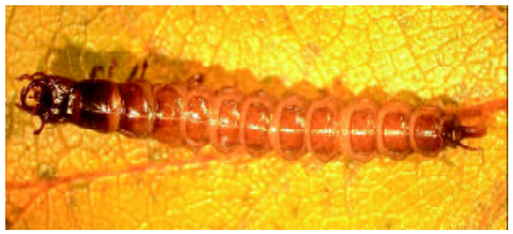

Figure 8. *Pterostichus melanarius* larva, identified from the autumn sampling. Approx 2cm in length.

only 10 carabid larvae were identified Numbers of adults (sampled using pitfall traps) were equally low. This may be due to: seasonal/annual variation, low numbers of carabids on the treatment plots generally and/or the dominance of other predatory groups such as Staphylinidae and spiders. Indeed, Staphylinidae adults and juveniles were the dominant arthropod predators and are currently being identified to species.

They were significantly more abundant in the Nitrogen + Lime treated plots compared to biocide and control plots.

Spiders were also prevalent, with Linyphiidae (money spiders) accounting for over 90% of the individuals. *Oedothorax retusus* and *Tiso vagans* were the most abundant species in the spring with *Allomengea scopigera* dominant in the autumn. A total of 29 species have been identified so far. Species abundance, richness and evenness measures were lower in the biocide treated plots.

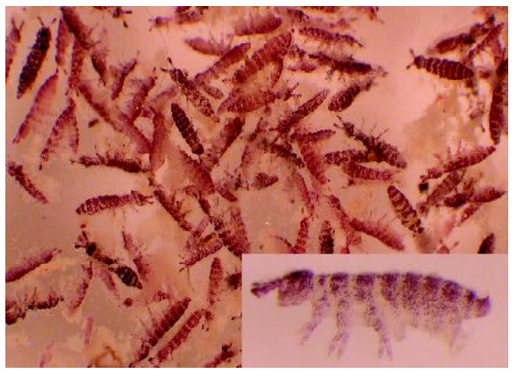

Figure 9. *Ceratophysella denticulata*, the dominating epigeic collembolan on biocide treated plots in Spring samples. Theindividual in the insetis approx. 1.6 mm in length.

Analysis of arthropod herbivore numbers has begun. We've found that slugs are very common on the site. Although no treatment differences were found in slug abundance from the spring sampling (using 'defined area traps'), by the autumn, numbers of *Deroceras reticulatum* were significantly higher in the biocide plots, which is assumed to be a response to the reduced predator numbers in this treatment.

In an addition to the project specifications, we have also examined springtail and mite abundance (aided byVicky Chapman, CAER). Although springtail numbers are higher in biocide plots, initial analysis of epigeic (i.e. feeding near the surface) species has revealed differences in species composition between the treatments, with one species accounting for over 95% of the population in the biocide treatment (Fig.9).

Although there are further experimental analyses to complete, emerging evidence suggests that treatments are showing definite changes in predator assemblages and that this is influencing herbivore communities.

Reviewing modelling strategies for the Programme submitted by Andy Meharg

The objective of our grant was to review modelling strategies for the NERC Soil Biodiversity Programme. This involved:

- Conducting a detailed review of existing soil carbon flux models
- Liaising with experimental research groups to ascertain their modelling requirements
- Liaising with experimental groups to ascertain the nature of the data they collected and the applicability of this for modelling purposes
- Making recommendations to the Soil Biodiversity Steering Committee concerning future modelling strategies within the programme.

From a survey of the literature we ascertained that the most suitable modelling approaches were those developed by Prof. Bill Hunt of Colorado State University, USA. As part of the modelling review, Mark Toal visited Prof. Hunt to discuss the application of his model to the Soil Biodiversity programme.

Following completion of a detailed questionnaire on modelling requirements and the structure and nature of their data, all experimental research groups participating in the Soil Biodiversity Programme were visited. From this process we identified those groups which had data sets suitable for modelling purposes. In September 2000 we organised a meeting of experimental research groups to discuss modelling strategies within the Programme.

As a result of these activities we proposed a unified modelling approach to the Steering Committee, with pragmatic strategies for modelling the data collected in the Programme. A tender to build a soil ecosystem model, with particular emphasis on transfers of carbon in the system was subsequently let to a

Assessments of chitin decomposer diversity: the role of actinomycetes and other bacteria in C & N cycling in limed & unlimed grasslands by Martin Krsek

In an attempt to characterise the impact of improvement regimes on the functional diversity of the below ground microbial community we have conducted the first ever study of the molecular diversity of chitinase genes within a terrestrial microbial community This required the development of molecular detection techniques for use with total community nucleic acids.

A molecular tool kit consisting of Polymerase Chain Reaction(PCR) primers of varying levels of degeneracy was produced to detect bacterial and fungal chitinase genes in the family 18 glycoside hydrolases, within which the majority of microbial chitinases isolated and characterised to date have been classified. These enzymes are implicated in chitin breakdown in the environment. Primers able to detect chitinase (*chi*) genes from a wide range of gram-positive and gram-negative bacteria were successfully applied to community DNA and RNA. This allowed a snapshot of molecular diversity within community chitinase genes to be taken for estimation of treatment impacts. Temporal and spatial variation were also studied to analyse the data statistically. The focus of this study was primarily the bacterial populations but fungal *chi* genes were also targeted. Analysis of fungal chitinase amino acid sequences indicated the presence of five groups and it was possible to design degenerate primers for two of these.

Analysis of chitinase gene diversity was achieved by baiting techniques at the Sourhope field site to allow analysis of chitin turnover rates and correlate this directly with functional and taxonomic diversity in community nucleic acids. The impact of liming and sludging on chitin degradation and *chi* gene diversity was analysed. Chitin baits were buried in litter bags and sampled in July and September 2000. Analysis of the microbial community by molecular profiling indicated significant enrichment compared to the surrounding soil. This community was analysed for *chi* gene diversity. For bacterial targets, over 200 clones were analysed and over 60 unique clones were sequenced from ten libraries. The libraries were dominated by actinobacterial-like *chi* gene sequences supporting our hypothesis that this group makes a significant contribution to chitin breakdown.

Phylogenetic analysis revealed clades of sequenced clones grouping with *chi* genes found in species of *Arthrobacter* (type Arth 69 dominated in sludge libraries but was not found in control libraries; Arth 62 was most prevalent in the lime library), *Streptomyces* and *Stenotrophomonas* (found in lime and control libraries but not present in any sludge library). No sequences bearing similarity to chi genes in *bacilli* or *clostridia* were found. All sequences were clearly distinct from any of those recovered from aquatic environments as presented in a published study (Metcalfe *et al.*2002). No significant difference in sequence diversity was found between control and limed plots but sludge application resulted in a significant reduction of diversity.
Analysis of chitin turnover and potential enzyme activity showed that the sludge treatment gave significantly higher turnover rates with a good correlation between weight loss and enzyme activity. Evidently sludging may improve fertility by increasing activity of key chitinolytic bacterial groups but at the expense of functional diversity.

Community analysis of the chitin bait colonists by 16S rRNA clone libraries revealed actinobacterial colonists identifying with members of the genera *Streptomyces*, *Rhodococcus* and eubacterial groups *Stenotrophomonas*, *Cellovibrio* and *Prosthecobacter*. The majority of 18S rRNA fungal clones were similar to *Chaetomium* sp. and *Mortierella* sp. Members of *Chaetomium* are common cellulolytic soil fungi.

We have initiated the first study of fungal *chi* gene diversity in situ. Primers for Group 1+2 and Group 2 were used to create fungal *chi* gene clone libraries using the same DNA prepared for the study of prokaryotic chitinases. Clones obtained using

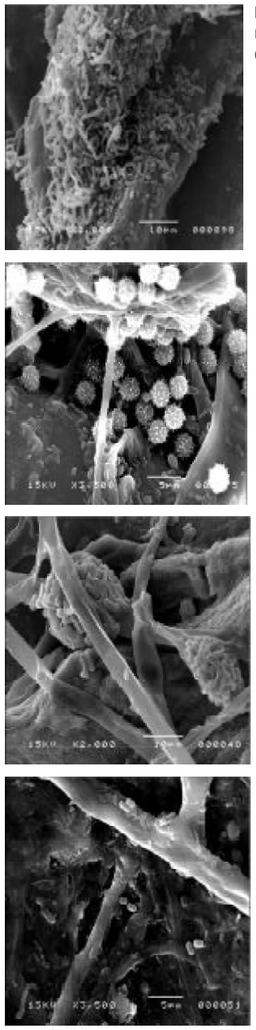

Fig 10 Scanning electron micrographs of microbial colonization of chitin in litter

the primer pair for Group 2 showed low diversity, the majority showing some similarity to *chi* genes from *Coccidioides immitis, Ajellomyces* spp. and *Emericella nidulans*. More diverse clones were obtained using primers for Group 1+2 and sequences had some similarities with *chi* genes found in *Ajellomyces, Aphanocladium*, *Hypocrea, Metarhizium* and *Trichoderma* species.

Through this novel work we have successfully studied the effect of a variety of soil management practices on communities of chitin decomposing bacteria and fungi.

Williamson, N. Brian, P, Wellington, E.M.H. (2000). Molecular detection of bacterial and streptomycete chitinases in the environment. Antonie van Leeuwenhoek 78: 315-321.

Krsek, M. Wellington, E.M.H. (2001). Assessment of Chitin Decomposer Diversity within an Upland Grassland. Antonie van Leeuwenhoek 79: 261-267.

Krsek, M. Metcalfe, A. Williamson, N. Wellington, E.M.H. (2001). Approaches to studying the molecular diversity of chitinase genes in soil. In: Chitin Enzymology 2001, R.A.A.Muzzarelli, ed., Atec, Italy, 2001: 361-272.

Metcalfe A.C. Krsek, M. Gooday, G.W. Prosser, J.I. Wellington, E.M.H. (2002). Molecular analysis of a bacterial chitinolytic community in an upland pasture. Appl. Environ. Microbiol. 68: 5042-5050.

Earthworm diversity and the integration of physical, biochemical and microbial functions by Hannah Bishop

Our projects at Sourhope explored the interactions between earthworms and soil microbial processes (Mark Pawlett, UeL), organic matter transformations (Hannah Bishop, Stirling) and physical structure of the soil (Christian Spring, Stirling). A field mesocosm with artificially manipulated earthworm treatments was established on a hillside adjacent to the main Sourhope site, using limed and unlimed soil. Half the soil was disturbed and half was left with its structure intact. The soil was reburied in plastic boxes, and left in place for 18 months (Fig. 11).

Figure 11. Reburied mesocosm boxes with mesh covers

The inoculated earthworm species were not consistently more abundant in inoculated boxes. *Lumbricus rubellus* was significantly more abundant in unlimed inoculated soil but not in limed soil. Conversely, significantly more *Allolobophora chlorotica* were found in limed inoculated boxes compared to unlimed boxes. The most abundant species found in unlimed soil was the uninoculated species, *Dendrodrilus rubidus*. *A. chlorotica* and *D. rubidus* were co-dominant in the limed treatments. The survival of the inoculated *L. terrestris* was uniformly poor.

At the outset of the field mesocosm experiment, an RDA analysis showed that variation in the depth of the LF soil horizon was the most important factor accounting for the spatial variation in earthworm species abundance. After 18 months however, liming was the factor that explained the most variation in spatial distribution.

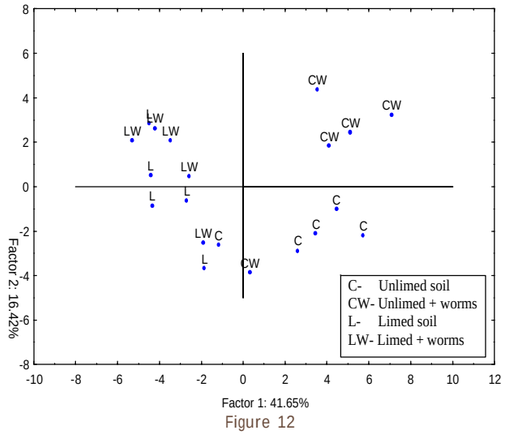

Figure 12

Laboratory microcosms with all three species inoculated were used to investigate the impact on soil microbial processes. Figure 12 shows a Principle Components Analysis of data from these microcosms. There is a clear limed-unlimed separation along the first ordination axis suggesting that principal component 1 is related to liming. The observed grouping of the unlimed earthworm-amended samples also shows a split of earthworm amended against non-amended microcosms suggesting that PC2 is related to earthworm additions. The effect of earthworms is less obvious in the limed samples, but there is a possible grouping of the earthworm-amended microcosms to the top of the limed group within the ordination plot. This indicates that liming causes a clear shift of the microbial community. Earthworms cause a population shift, but to a lesser extent.

Five different measures were used to assess the impact of earthworms on soil structure:

• Group1 (Void space measures): Saturated hydraulic conductivity,bulk density, void space as measured using image analysis.

• Group2 (Structural stability measures): Aggregate stability, excremental pedofeatures as quantified by using micromorphological techniques.

The group 1 results show that earthworms affect the porosity of soil, indicated by both significant decreases in bulk density and concomitant increases in the proportion of large voids (>500mm^2) to small voids (<500mm^2).

This tends to indicate that rather than simply increasing porosity, earthworms have a re-organisational effect on void space by creating more large voids. Group 2 results show that despite a high degree of bioturbation in samples, the casting activities of earthworms do not significantly affect soil structural stability.

Our mesocosm studies at Sourhope have helped us to elucidate the effects of liming on earthworm populations. Through microsocm experiments we have also advanced our understanding of how earthworms affect microbial processes in soil.

The role of Hygrocybe species in decomposition by Gareth Griffith

The aim of this project is to investigate the effect of nutrient treatments on the distribution of basidiocarps of *Hygrocybe* species (waxcap fungi) and to establish what role they play in soil nutrient cycling. Waxcaps are amongst the most visible components of the soil biota due to their often large and brightly coloured basidiocarps (Figure 13). In Europe, these fungi are associated with oligotrophic semi-natural grasslands (curiously found in woodlands in most other parts of the world), post-war agricultural intensification has led to a precipitous decline in the abundance of these habitats, with only some 200 ha remaining in the Netherlands. Although nutrient-poor grasslands are still widespread in the UK, several *Hygrocybe* species are the subject of Biodiversity Action Plans and information is urgently required about their habitat requirements.

Fig 13. *Hygrocybe conica* at Sourhope.

Waxcaps are difficult organisms to culture, and due to their large size (single individuals often covering several square metres), they are not amenable to study in micro/mesocosms. Thus we are applying a number of novel approaches to their study, including the measurement of stable isotope natural abundance, ^{13}C /^{15}N pulses, PCR-based approaches to assessing mycelial distribution in the soil and genetic relatedness, as well as fine-scale mapping of basidiocarp distribution using differential GPS.

Mapping of basidiocarp abundance for 11 species in 2001 and 2002 has shown that nutrient treatments (especially liming) severely affect fruiting of these fungi with more than 95% fewer basidiocarps found on limed and limed nitrogen plots. Distribution of basidiocarps is across the site is distinctly non-random. Using GIS software we are attempting to correlate basidiocarp distribution (mapped to 50cm squares) with the microtopographic, hydrological and soil maps produced by Mick Whelan and Patricia Bruneau from Stirling. The non-random distribution of waxcap basidiocarps is also due to the fact that they are probably the largest soil organisms present

at Sourhope and thus it is important to establish the extent of individual mycelial genets. Using the microsatellite-based ISSR method, it is already clear that some genets extend over several metres and that some extend beyond treatment blocks.

Analysis of stable isotope natural abundance has shown that *Hygrocybe* species are distinctly enriched for ^{15}N and depleted for ^{13}C not only in comparison with basidiocarps of several saprotrophic taxa from Sourhope (e.g. C*ystoderma*, *Mycena*, *Entoloma*) but also with respect to data from various studies in other habitats (figure 14). Furthermore analysis of natural abundance patterns from fruit bodies of members of the *Geoglossaceae* and *Clavariaceae* (earth tongues and fairy clubs) showed this group to be similarly enriched for ^{15}N and even more depleted for ^{13}C .

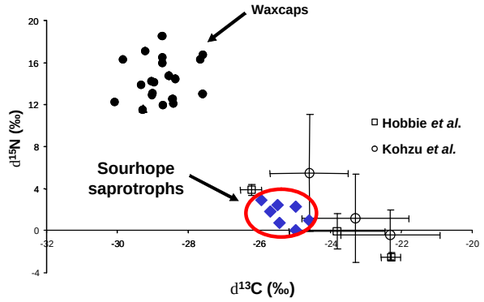

Fig.14. ^{13}C /^{15}N natural abundance levels in fruit bodies of *Hygrocybe* spp. from Sourhope. Also shown are values for saprotrophic fungi, as well as data from similar studies involving woodland fungi (Hobbie *et al*., 2001. New Phytol., 150: 601; Kohzu *et al*., New Phytol., 144: 323)

This suggests that these three groups of fungi, taxonomically unrelated but all characteristic of oligotrophic grasslands, have a similar distinctive mode of nutrition. Despite the clear differences between these and other fungi, it is perplexing to note that ^{15}N values differed by up to 8.9‰ between two basidiocarps of *H. pratensis* from control plots.

It is unwise to infer too much from natural abundance data and as such we are conducting pulse-labelling experiments with $^{13}CO_2$, and also with ^{15}N-labelled litter buried at different depths.

Griffith, G.W. Easton, G.L. and Jones, A.W. (2002). Ecology and Diversity of Waxcap (*Hygrocybe* spp.) Fungi. Botanical Journal of Scotland, 54:7-22.

Biodiversity of saprotrophic fungi of grassland in relation to their function
by Clare Robinson, Janie Pryce-Miller & Lewis Deacon

Saprotrophic (decomposer) fungi often underpin nutrient cycling in ecosystems but are sensitive to disturbance, pollution and environmental change. NERC CASE studentships plus an associated grant held between the Department of Life Sciences, King's College, University of London (CHR and Brian W. Bainbridge) and CEH Merlewood (Juliet C. Frankland) aimed to improve our understanding of the diversity and function of these organisms.

Janie Pryce Miller's studentship was 'Taxonomic biodiversity and community structure of grassland saprotrophic fungi: use of molecular methods'. The main aims were (1) to characterise, in unimproved and 'improved' upland grassland soils, the broad picture of taxonomic biodiversity of saprotrophic fungi and (2) to identify, using molecular techniques, the niches in which the mycelium of key fungal species occur in soil.

Two principal outcomes were that the species found in this study were typical of communities of decomposer fungi in grasslands, although separate associations of fungi were found in standing-dead plant material, soil and for fruiting basidiomycetes. Showing that the microfungi isolated were largely cosmopolitan, the most frequently isolated species from both the standing-dead material and the soil samples were found to be common between 'improved' and unimproved upland grassland at Sourhope. Similarly, no microfungal species were isolated which were specific to one treatment or location, probably because of the short time lapse since the treatments were started. It was the structure (i.e. frequency of occurrence of particular species) of the microfungal community which differed between the control, lime and nitrogen plus lime plots. Species diversity was greatest in the lime-treated plots, and nitrogen application reduced species diversity. 'Key' species were chosen because they were isolated frequently and because of their potential enzymatic capabilities. In model systems, T-RFLP profiles of four of the 'key' fungi (*Cladosporium cladosporioides, Fusarium oxysporum, Penicillium hirsutum* and *Trichoderma koningii*) were successfully visualised for soil 'spiked' with added mycelium. The T-RFLP profile generated did differentiate between the fungal mycelium in pairs and for all four species en masse. Unfortunately, T-RFLP analysis on field soil samples failed at the stage of amplification of soil DNA extracts, even though amplification of Polymerase Chain Reaction products from native soil and litter extracts had worked on previous occaisions.

The aims of Lewis Deacon's studentship 'Functional biodiversity of grassland saprotrophic fungi' were (1) to characterise, in unimproved and 'improved' upland grassland soils, the functional biodiversity defined by substrate utilisation of saprotrophic fungi and (2) to determine whether there was a high degree of functional redundancy in decomposer fungal communities at Sourhope.

Fifty isolates, with equal numbers of frequently occurring and occasional species were selected by Lewis from fungal cultures obtained from Sourhope soil and standing-dead grass litter. They were screened for potential enzyme activity on solid media. As carbon sources in the media became more complex (e.g. from starch to cellulose to lignin), the number of fungal isolates able to use them decreased. A high degree of potential functional overlap was observed between the abundant and occasional species.

Results from these initial tests led to a reduced species list of twelve isolates, enabling a more in-depth study of the functional capabilities of each. These isolates were chosen, by linking in pairs, two isolates with similar functional abilities, one of which was abundant and one of which was occasional. The BIOLOG system was used to provide a detailed profile of the functional capabilities of each of the twelve isolates on 95 different carbon sources. From this work, even though an isolate was abundant in the field, it did not mean that it would have a wider range of functions, or a higher activity, than an occasional species. Experiments are under way on more natural substrata to test this hypothesis.

As early as 1982, Kjøller and Struwe stated that a full understanding of the role of fungi in an ecosystem is not reached through independent observations on numbers, biomass, lists of species or physiological groups, but only through combined investigations where the relative occurrence of the different groups of fungi is linked to their function, that is, activity or capacity for substrate utilisation. Over twenty years later, the need for insight into the functional role of fungi in ecosystems is still great, partly because of the inadequate description of fungal assemblages in relation to resources. We hope our study goes a significant way to match organisms, their location at the fine-scale and their activities together. Finally, it is apparent that this type of project is extremely time-consuming being three years work for each of two PhD students.

Kjøller A. and Struwe S. (1982) Microfungi in ecosystems: fungal occurrence and activity in litter and soil. Oikos 39: 391-422.

Effects of soil improvement treatments on bacterial community structure and function

by Neil Gray

In the early summer of 2000 we visited Northumbria Water's Hexham sewage works, relieved them of 1000 litres of anaerobically digested sewage sludge and lugged it to Sourhope. What on earth possessed us?

A direct consequence of the 1998 EU ban on disposal of sewage sludge at sea is that its application to agricultural soils is predicted to increase. Sewage sludge and other widely used soil improvement treatments (e.g. liming) are thought likely to have effects on bacterial function and diversity. With this in mind we have used molecular biological tools to characterize the response of particular components of the microflora to soil improvement treatments and have related these effects to measurements of key soil processes.

Figure. 15. An onerous and certainly not odourless task! Application of anaerobically digested sewage sludge to sub-plots at the Sourhope site in May 2000.

An unexpected but key observation was that despite changes in pH, nitrogen content, plant growth and soil processes such as ammonia oxidation and denitrification (Figure 16), soil improvement treatments had little effect on overall microbial activity as indicated by soil respiration data. Bacterial community structure also appeared to be relatively unaffected, in fact variation with time exceeded the effects of soil improvement treatments (Figure 16). These findings were all the more surprising since the perturbations imposed were rather extreme (application of double the recommended quantity of sewage sludge, and lime treatment that altered the pH by 3 units from 4 to 7).

Consistent treatment effects on autotrophic ammonia-oxidation and denitrification rates were however, apparent (Figure 16). Interestingly this was not reflected in the communities of ammonia-oxidizing bacteria (AOB). Selection of specific AOB by different treatments was only transient and the populations were otherwise highly variable. It was however apparent that AOB communities exposed to soil improvement treatments were more dynamic and variable than in soils that were left untreated. Despite considerable variation in the AOB community structure, treatment effects on ammonia oxidation rates were pronounced and prolonged (Figure 16). One explanation is that the AOB communities exhibit a degree of redundancy, since differences in AOB community structure did not relate specifically to ammonia-oxidation rates.

It is clear that our understanding of the relationship between bacterial community structure and soil processes is still rather basic, and simple assumptions about the effects of community composition on soil processes do not seem to hold. At a more practical level, this study also demonstrated that at least in Sourhope soil, increased release of carbon dioxide to the atmosphere may not result from sewage sludge or lime amendment. However all of the soil improvement treatments significantly enhanced denitrification and may potentially increase the production of nitrous oxide, a significantly stronger greenhouse agent than carbon dioxide.

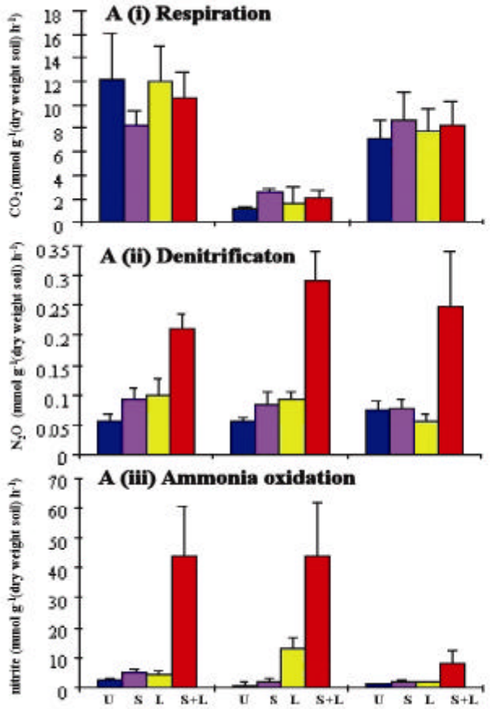

Figure 16 A+B. Soil processes (A) Rates of basal respiration (i), denitrification (iii), and ammonia oxidation (iii) in soil plots treated with sewage sludge (S), lime (L) and (S + L). Untreated control plots (U). B. Soil gas dynamics over two diurnal cycles measured at 5 cm depth in a Sourhope soil monolith by Membrane inlet mass spectrometry.

While Sourhope soils may not be typical and methods of measurement currently available may be imperfect, our data suggest that microbial community structure may not be the most significant factor in shaping many biogeochemical processes in soil. Whether this is also true of activities catalysed by organisms that are rare and where functional redundancy may be less significant (e.g. organisms involved in the degradation of a specific pollutant) remains to be demonstrated. With better *in situ* measures of soil processes and dynamics, such as that provided by membrane inlet mass spectroscopy (MIMS, Figure 16B) combined with molecular characterization techniques, these issues may be addressed.

What is the link between microbial diversity and soil resilience?

by Anne Glover

This project aimed to relate soil resilience to biodiversity by measuring changes in both microbial function (ability to metabolise a complex substrate) and microbial diversity after an applied stress. These parameters were assessed at three different scales (microcosm, mesocosm and field).

In microcosm studies, Sourhope soil was manipulated to generate microbial communities of different diversity in order to determine whether resilience to stress was related to the initial microbial diversity of the soil. Diversity was manipulated in 2 ways:

- Deconstructive approach: progressive fumigation to reduce diversity
- Constructive approach: sterilisation of soil followed by reinoculation of dilutions of the initial microbial population.

For mesocosm studies, the experimentally altered soils from the microcosm study were planted with a dominant grass (*Agrostis capillaris)* from the Sourhope site. This allowed the assessment of impact of a rhizosphere on function and diversity.

The field scale samples were taken from Sourhope plots receiving different treatments and functional analysis under both acute and chronic stress were measured. This allowed the assessment of additional treatments on resilience.

Soil resilience was assessed in the microcosms (both constructive and deconstructive but with matching biomass) by the application of a transient 40°C heat stress and a chronic application of copper amendment. Function was assessed subsequently by measurement of decomposition rates (over 24 h) of powdered grass from Sourhope over a 28 day recovery period. Microbial diversity was measured by using molecular techniques.

All samples recovered function after acute heat stress. The functional resilience of fumigated soils to heat stress increased with fumigation time, with the soils fumigated for 24 h displaying the greatest recovery in function. The sterilised soils did not display significant differences in resilience to heat. In contrast, there was little recovery in function in response to chronic stress (application of copper).

Both treatments resulted in distinct shifts in microbial community structure. The shifts in microbial community structure, and by inference from previous studies a reduction in overall microbial diversity, were associated with a reduction in functional stability.

In the mesocosm experiments temporal shifts in functional resilience exceeded any effects of planting grass.

In the field experiments, samples were taken from three of each of the control, reseeded, sewage-amended, biocide and nitrogen + lime treated plots. The resilience of these soils was measured using the same stress assays as the microcosm and mesocosm samples.

Soil samples from all the treated plots were less resilient to heat stress than soil from control plots. Despite spatial variation in soil characteristics, e.g. nitrate and dissolved organic carbon, soil from the Sourhope plots showed marked differences in resilience by treatment type. The initial resistance of function to stress was not predictive of recovery of function over time and emphasises the importance of measuring resilience.

No direct link has been inferred between soil resilience and microbial diversity from this study as the resilience of soil to stress is dependent on a range of factors. Soil resilience varies according to the type and duration of stress applied, microbial community structure, soil characteristics and treatment regimes. The mesocosm study also demonstrated temporal changes in soil resilience. The assessments of soil resilience in microcosm, mesocosm and field trials have demonstrated that the assay developed in this project is robust and applicable at a range of scales. However, so far, we have not been able to use microbial diversity, or community structure as a predictor of potential resilience to stress.

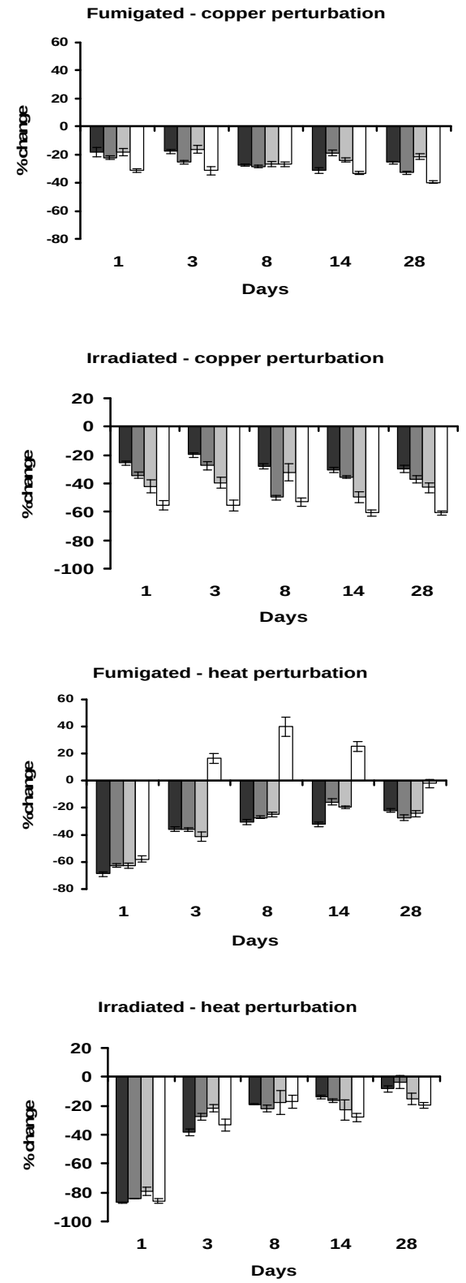

Figure 17. Microcosm resilience. The percentage change in respiration (CO_2 evolution over 24 h), from powdered grass added to soils, 1, 3, 7, 14, or 28 days after perturbation by copper or heat, compared to an unperturbed control. The soils were originally treated by fumigation for 0 (n), ½ (n), 2 (n) or 24 h (o) (top) or irradiation and reinoculation with a 10^{-2} (n), 10^{-4} (n), 10^{-6} (n) or 10^{-8} (o) dilution of a soil suspension (bottom). Microbial diversity was reduced with increasing fumigation time or dilution.

Grassland responses to altered soil faunal community composition by Mark Bradford

Numerous investigators from both within and outside the Soil Biodiversity Programme combined forces with the Ecotron Research Team to determine the effect of experimentally manipulating soil faunal community composition on grasslands under stable conditions and after an experimental perturbation. We took advantage of the precise environmental control afforded by the Ecotron facility to establish and then maintain 15 model Sourhope ecosystems for 18 months. Three treatments were applied to form a gradient of increasing soil biotic functional complexity: a) microbiota only; b) microbiota and mesofauna; c) microbiota, meso- and macrofauna.

Figure 17. Sampling in the Ecotron

The impacts of the treatments on carbon flux, and microbial and plant community composition and abundance under stable conditions were reported in Science 298: 615-618 (2002).In brief, we showed that plant community composition, microbial and root biomass, decomposition rate, and mycorrhizal colonization, were all markedly affected. However, two key ecosystem processes, above ground net primary productivity and net ecosystem productivity, were surprisingly resistant to these changes. The findings demonstrated that marked effects on ecosystem processes of changes in faunal size-class composition, observed in simple experimental systems, do not necessarily manifest themselves within complex communities. Further, the reason for the observed resistance may be that positive and negative faunal-mediated effects in soil communities cancel each other out, causing no net ecosystem effects. This hypothesis was put to the test after the fate of isotopically labelled CO_2, introduced to the communities via photosynthesis, was followed into the model grasslands, then within the communities themselves and then finally back out again (as respired CO_2).

The core Team would like to thank all of the collaborators on this project – their input has made the research an even more exciting, interesting and rewarding venture.

Bradford, M.A. Jones, T.H. Bardgett, R.D. Black, H. Boag, B. Bonkowski, M. Cook, R. Eggers, T. Gange, A.C. Grayston, S.J. Kandeler, E. McCaig, A.E. Newington, J.E. Setala, H. Staddon, P.L. Tordoff, G.M. Tshcerko, D. Lawton, J.H. (2002). Impacts of soil faunal community composition on model grassland ecosystems. Science, 298: 615-618.

The diversity and distribution of the arbuscular mycorrhizal fungi in the Sourhope experiments by JPW Young

Arbuscular mycorrhizal (AM) fungi are biotrophic symbionts colonizing about two-thirds of land plant species and found in all ecosystems. They are of major importance in plant nutrient supply and their diversity is suggested to be an important determinant of plant community composition. The diversity of the AM fungal community composition in the roots of two plant species (*Agrostis capillaris* and *Trifolium repens*) that co-occurred in the Sourhope grassland control plots was characterized using molecular techniques (Vandrnkoornhuyse, *et al.*2002). We analysed the small subunit (SSU) ribosomal RNA gene amplified from a total root DNA extract using AM fungal-specific primers. The diversity found was high: a total of 24 different phylotypes (groups of phylogenetically related sequences) colonized the roots of the two host species. Phylogenetic analyses demonstrate that 19 of these phylotypes belonged to the *Glomaceae*, three to the *Acaulosporaceae* and two to the *Gigasporaceae*.

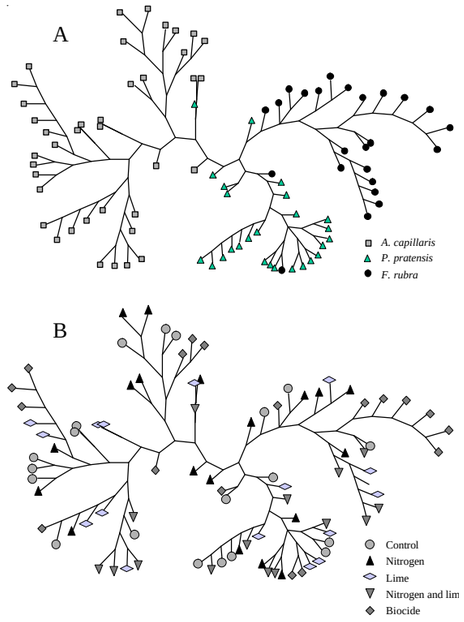

Fig. 19. The similarity among AM fungal communities displayed as the shortest unrooted maximum parsimony tree. Each terminal corresponds to the AM fungal community in a single root; the closer they are the more similar is their profile of T-RFLP fragments. The same tree is shown labelled (A) by host species and (B) by soil treatment.

Our study reveals clearly that the AM fungal community colonizing *T. repens* differed from that colonizing *A. capillaris*, providing evidence for AM fungal host preference. In addition,our results reveal dynamic changes in the AM fungal community through time.

The approach just described provides detailed information about the AM fungal genotypes present, but each sample requires a large investment of time and resources. We therefore developed a more rapid method to characterise the AM population in a root. using the terminal-fragment length polymorphism (T-RFLP) strategy. The diversity of AM fungi was assessed in roots of three grass species (*Agrostis capillaris, Festuca rubra, Poa pratensis)* that co-occurred in the same plots of the field experiment. The impact of different soil amendments (nitrogen, lime, nitrogen and lime) and insecticide application on AM fungal community was also studied. The level of diversity found in AM fungal communities using the T-RFLP strategy was consistent with that found using the cloning method described above. Our results clearly confirmed that an AM fungal host-plant preference exists (even between different grass species). AM communities colonising *A. capillaris* were statistically different from the others. Although grass species evenness changed in response to the nitrogen and/or lime amendments, AM fungal community composition in roots of a given grass species remained stable. Conversely, in plots where insecticide was applied, we found higher AM fungal diversity and, in *F. rubra* roots, a statistically different AM fungal community. It would be mere speculation at this stage to suggest that this might relate to changes in fungal-feeder populations.

Overall, we have shown that the AM fungal community at Sourhope is diverse, and that the different fungal types have different ecological behaviours, both in terms of host preference and in response to an imposed change.

Vandenkoornhuyse, P. Husband, R. Daniell, T.J. Watson, I.J. Duck, J.M. Fitter, A.H. Young, J.P.W. (2002). Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Molecular Ecology 11: 1555-1564.

Mycorrhizal functioning at Sourhope
by Dave Johnson

Arbuscular mycorrhizal (AM) fungi are the dominant symbiotic micro-organisms in upland grasslands but our knowledge of their functioning has been limited by reliance on simplified experimental systems such as the use of pot-based sand culture studies. A major feature of our research programme, therefore, was to develop and apply new methods to enable functional studies of AM fungal communities to be undertaken either under field conditions *in situ*, or in blocks of turf removed from the field.

We developed mesh-walled cores that are filled with non-sterile plant-free soil and inserted into turf (Fig. 20). The soil within the cores becomes rapidly colonised by external AM mycelium because the mesh does not restrict its growth, but it does prevent in-growth of roots. Duplicate control cores are rotated to snap hyphal connections so that the soil within them contains no active mycelium (Johnson *et al.* 2001).

We used the cores to study the role of AM fungal mycelium in phosphorus uptake by plants. We injected radioactive orthophosphate (^{33}P) into cores filled with soil that had previously been inserted into turfs. Within 48h, the plants surrounding unrotated cores had 10-fold greater shoot ^{33}P concentrations than those surrounding rotated cores. Strikingly, when core rotation ceased, the shoot ^{33}P concentrations equalised between the two core systems, and when it was re-

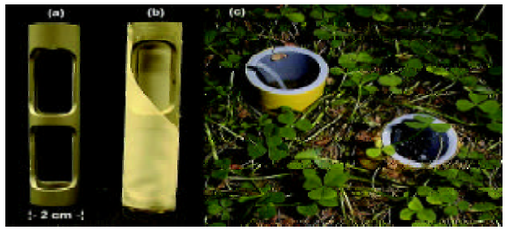

Figure 20. Cores developed for functional studies of external AM mycelium; a) prior to addition of mesh, b) cut-away of mesh, c) added to a turf of T. repens with nylon injection port.

started, they were reduced.

In collaboration with Phil Ineson and Nick Ostle, we undertook the first quantification of carbon movement from plants through AM mycelium in field conditions, by use of ^{13}C pulse labelling. Approximately 5% of the C fixed by plants was consumed by respiration from external AM mycelium in the first 24h after labelling (Johnson *et al.* 2002a). Parallel laboratory studies using $^{14}CO_2$ found that carbon transfer into rotated cores where AM hyphae were severed was reduced by 90% compared to unrotated cores (Johnson *et al.*, 2002b).

Further collaborative work with Phil Grime, Mark Bailey, Andy Whiteley, Philippe Vandenkoornhuyse and Peter Young investigated how plant community composition affected microbial activity and diversity. We found that plant communities supporting an active AM mycelial network reduced both soil bacterial diversity (Johnson *et al.* 2003) and the diversity of AM fungi colonising the roots of establishing seedlings.

The work described above has led to a second Soil Biodiversity award (with Liz Wellington and Martin Krsek) studying interactions between C cycling,AM mycelium, microarthropods and bacteria.

Johnson, D. Leake, J.R. Read, D.J. (2001). Novel in-growth core system enables functional studies of grassland mycorrhizal mycelial networks. New Phytol. 152: 555-562.

Johnson, D. Leake, J.R. Ostle, N. Ineson, P. Read, D.J. (2002a). *In situ* $^{13}CO_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelium to the soil. New Phytol. 153: 327-334.

Johnson, D. Leake, J.R. Read, D.J. (2002b). Transfer of recent photosynthate into mycorrhizal mycelium of an upland grassland: short-term respiratory losses and accumulation of ^{14}C. Soil Biol. Biochem. 34: 1521-1524.

Johnson, D. Booth, RE, Whiteley AS, Bailey MJ, Read DJ, Grime, JP & Leake JR. (2003) Plant community composition affects the biomass, activity and diversity of soil microorganisms in reconstituted calcareous grassland. European Journal of Soil Science 54 (In press).

Soil mesofauna and carbon cycling
by Nick Ostle

Upland soils in grassland and peatland ecosystems are important reservoirs of terrestrial carbon that have considerable potential **as sinks and sources of atmospheric CO_2**. Understanding the nature and extent of biological regulation of these below-ground carbon stocks is therefore a priority. Soil faunal foodwebs have traditionally been considered to be dominated by detritivorous organisms that rely on the decomposition of dead organic matter for sustenance. It is clear, however, that in montane grassland systems, where roots can account for over 50% of live plant

Figure 21. Mobile laboratories at Sourhope

biomass, recent root derived carbon inputs into the rhizosphere may represent a significant source of organic substrates.

In this three year research programme a series of experiments examined the role of plant root inputs (i.e. rhizodeposited exudates, cells and root death) as possible substrates for soil fauna groups including earthworms, enchytraeids, nematodes, mites and collembola. This required the development of $^{13}CO_2$ pulse labelling equipment (Figure 21)using *in situ* photosynthesis to introduce the ^{13}C tracer into the soil foodweb.

Key findings from these studies made at the Sourhope field site show that, far from being dependant on decaying organic matter, faunal groups such as earthworms, enchytraeids, nematodes, collembola and mites were rapidly exploiting organic matter containing pulse ^{13}C . It became clear that, although *Allolobophora chlorotica* earthworms represented the greatest contribution to soil animal biomass, other organisms with less biomass accounted for most pulse derived ^{13}C per square metre Field $^{13}CO_2$ pulse-chase experiments also allowed us to determine that liming had an important effect on below-ground biological activity producing an increase in initial rates of ^{13}C return to the atmosphere as CO_2 possibly as a consequence of changes in rhizosphere activity. Controlled experiments using a novel ^{14}C depleted carbon dioxide tracer approach enabled us to determine the turnover of carbon in the extraradical mycelia of arbuscular mycorrhizal fungi. Results of accelerator mass spectrometric analyses made on fungal tissues showed that individual *hyphae* have a turnover time of 5 to 6 days.

Taken together these findings confirm that recent root carbon inputs are readily accessible to and exploited by organisms previously considered as detritivorous. This means that changes in climate and landuse that affect plant below ground productivity, faunal diversity and biomass will be important governors of soil carbon transformations and release as greenhouse CO_2 .

A key part of the research project has involved collaboration with other research groups (Stirling, Sheffield, MLURI, IGER) that used the ^{13}C tracer approach to look at aspects of plant and soil carbon dynamics. As a result, the ^{13}C tracer approach now constitutes a central component of the NERC Soil Biodiversity Phase II research programme.

The Soil Biodiversity Database

by Lynne Irvine

The data collected by the Soil Biodiversity Programme has been migrated to an Oracle relational database, designed to hold information on all aspects of the research activities. Currently, it contains site management details gathered over the lifetime of the programme, such as site treatments, experimental setups, site visits, and records of when measurements and samples were taken. It also holds baseline datasets which include the soil and vegetation surveys, and data from the Automatic Weather Station (Figure 22).

Figure 22. Automatic Weather Station at Sourhope

The database is now being extended to incorporate results datasets submitted by Phase 1 projects. Datasets are integrated through the site visit, sampling and measurement identifiers, which were allocated on site to establish a specific time and location for every sample and measurement taken. Links between project datasets may also be made where datasets share certain characteristics such as treatment, experiment or soil type. When complete, the database will be a valuable resource for soil scientists.

To provide access to the data, a web based "Data Discovery and Download" facility is being developed and planned for completion by December 2004. The Data Discovery interface will use keyword and free text retrieval to extract information from the database. It will provide secure and controlled access to the database, enabling only authorised users to search and download datasets.

Information about each project and its research activities will be openly accessible to the wider community, from details held in the metadata tables, but they will be denied access to the physical data. All access to the database will be monitored and reported to the project investigators.

Farewell to Mike!

by Bill Heal

Mike Hornung retires from the Centre for Ecology & Hydrology at the end of March. We will miss him, that life-long advocate of soil science,'The Last of the Black Arts'. Mike Hornung is a soils man through and through. He understands the way in which that medium is the interface between the past and the present and is the key to the future. He enjoyed and understood the magic in its diversity but never ignored the wider ecological, environmental and social context. He has his biases though and it is noticeable that the hills have never been far from his workplace. His PhD was firmly based on the soils of Moor House the North Pennines, but his pedology was linked to the extensive soil biology of that site. Then he moved to the Nature Conservancy in Bangor, joining a thriving Soil Section which he eventually led. He plied his trade in and gained an intimate knowledge, plus many friends, in nature conservation, upland forestry and hydrology. Soils are a hobby as well as a profession and the Welsh Soils Discussion Group provided social as well as professional contacts.

Mike's appointment as Head of Merlewood in 1987 was a natural move from Bangor, cementing long-standing links and similar 'cultures'. His soils knowledge and his gregarious character enabled him to contribute easily with the diversity of research at Merlewood and to the management of the Institute of Terrestrial Ecology in the North. He naturally maintained a wide range of connections - the number of carefully laid piles of paper became a feature of his office - and the bane of his PA's life! His wide experience, creative approach, sound judgement and outgoing personality made him a valuable asset. He was drawn into a wide variety of topics and organisations nationally and internationally, including the Department of the Environment, acid rain and critical loads in Europe, Agricultural and Fisheries Research Council Soils Committee, Environmental Change Network and of course the Soil Biodiversity Programme. Throughout all the ups and downs of an extensive and intensive career, Mike has been a great colleague and friend to many, always ready to discuss research, politics or sport, even the musical attributes of the didgeridoo! We will certainly miss him. Long live The Black Art and its practitioners.

This issue of the Soil Biodiversity newsletter first appeared in March 2003. It has been reissued to coincide with the Programme's final events marking the completion of the Programme in 2004.

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Programme Manager
Andrew Sier
Soil Biodiversity Programme
Centre for Ecology & Hydrology
Lancaster Environment Centre
Library Avenue
Lancaster LA1 4AP

Editor(to whom copy should be sent)
Miss Rebecca Pinder
Tel: 01524 595800(switchboard)
Fax: 015395 61536
E-mail: soilbio@ceh.ac.uk

Website: http://soilbio.nerc.ac.uk