Biological Diversity and Ecosystem function in Soil

Soil Biodiversity

NERC Thematic Programme

Predatory mesostigmatid soil mite, CEH Merlewood

Newsletter - Issue number 8 *May 2002*

Soil Biodiversity Second phase awards

Twenty six standard and small grant proposals were submitted to Phase 2. The Steering Committee recommended funding of eight of these proposals, and the awards have now been confirmed.

Prof Mark Bailey, CEH Oxford
Influence of Sourhope biodiversity treatments on the carbon flow and functional resilience of key bacterial taxa in the rhizosphere of grassland soils.

Dr Ian Grieve, University of Stirling
Flow paths and rates of labelled carbon transfer within the spatial organisation of an upland grassland soil.

Dr Gareth Griffith, University of Wales
Fine scale analysis of Hygrocybe spp. in semi-natural grasslands and elucidation of their role in decomposition processes.

Prof Philip Ineson, University of York
Carbon transfers at Sourhope - from plant to soil organic matter.

Dr Jonathan Leake, University of Sheffield
Carbon flow through mycorrhizal mycelial systems to soil microbial populations - their impact on microarthropod diversity.

Dr Philip Murray, IGER
Diversity and functional role of predatory beetles and their prey in the Sourhope ecosystem.

Prof James Prosser, University of Aberdeen
How does rhizosphere carbon flow drive soil biodiversity?

Prof Elizabeth Wellington, University of Warwick
Provision of a molecular archive for microbial diversity within treatment plots at Sourhope.

Goodbye SID hello 'Spike'

During the first phase of the NERC Soil Biodiversity and Ecosystem Function thematic programme a mobile laboratory was built and developed, for use in a soil fauna carbon cycling study. The Stable Isotope Delivery Lab (SID) functions by removal and simultaneous replacement of atmospheric CO_2 with ^{13}C labelled CO_2. This $^{13}CO_2$ and air mix is then supplied to transparent acrylic chambers to be assimilated by natural photosynthesis. We, at CEH and SIF, worked in collaboration with a number of research groups using these field-based ^{13}C stable isotope tracer technologies on investigations following ^{13}C labelled photosynthate flow through plant tissues, soil microbiota, mesofauna and back to the atmosphere as CO_2

Phase 2 award holders met in London in October 2001 to exchange information on their proposed projects and discuss their isotopic requirements, where relevant, for the $^{13}CO_2$ pulse labelling at Sourhope. A contract has subsequently been let to Dr Nick Ostle at CEH Merlewood to provide the pulses (or 'spikes') in collaboration with these projects and the laboratory has now been upgraded to extend its range. Discussions between Nick, Ostle, Niall McNamara, Graham Burt-Smith from Sourhope and the award holders have agreed the following provisional schedule of pulses in the 2002 season.

June

Week	1	Grieve at al. short pulse
	2	Leake et al. short pulse
	3	Ineson et al. short pulse

July

Week	1- 3	Bailey et al. Prosser et al. long and short pulse
	4	Murray et al. short pulse

September

Week	2	Ineson et al. short pulse
	3	Griffiths et al. short pulse
	4	Leake et al. short pulse

We have limited flexibility with regards to time so please, no rain dances!
Please contact Nick Ostle (no@ceh.ac.uk) at CEH Merlewood for further details

Mike Hornung, CEH Merlewood

Nick Ostle & Niall McNamara, CEH Merlewood

Website: http://www.nmw.ac.uk/soilbio

Microarthropods at Sourhope

The microarthropods are a group of soil mesofauna that contains the mites and Collembola (springtails). Although these animals are very small - typically measuring less than 1 mm in length - they are known to have many beneficial effects on decomposition and nutrient cycling in soil. Very little is known about what controls their diversity in soils, so one of the key aims of our research group at Lancaster was to identify how many species were present at Sourhope and how this diversity varies across a range of environmental productivity gradients.

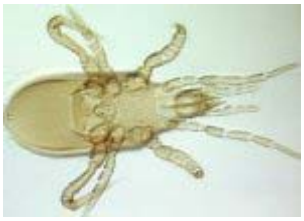

Plate 1: *Lysigamasus truncus*, a predatory mite from Sourhope

Since the identification of these animals is difficult and time consuming, we decided to do an intensive sample of all treatments on one date in August when population densities were likely to be highest. At the same time, we measured a range of other soil and plant properties to obtain data that may help explain the observed diversity of the fauna. To help with the identification of animals, Lisa Cole attended a course on Introductory Acarology at Ohio State University, and also spent time with Henk Siepel, at Wageningen, looking at the mites from Sourhope.

We have found that the diversity of microarthropods at Sourhope is very similar to other grassland sites being rather species poor, and dominated by just a handful of mite species such as *Scheloribates laevigatus* and *Liebstadia similis*. In total, we have recorded 19 species of Oribatid, 14 species of Mesostigmata (Plate 1) and representatives from 5 families within the Prostigmata.

Plate 2: *Lepidocyrtus lignorum*, a collembolan from Sourhope showing the 'furca' or springing organ that gives them their alternative name 'springtail'

We have found 12 species of Collembolan (Plate 2), but as with the mites this community is dominated by two species, *Folsomia quadrioculata* and *Protaphorura bicampata*. There appears to be very little difference in the diversity of these animals between the experimental treatments. In view of this, we are now exploring our additional experimental manipulations that impose severe physical disturbance as well as stresses on this system.

Our field manipulation experiment (Plate 3) was set up in November 1999. It consists of plots containing crossed, continuous gradients of fertiliser addition (0, 60, 120, 180 and 240 kg N ha^{-1}) and disturbance intensity (0%, 25%, 50%, 75% and 100% ground cover disturbed).

We have been looking at the influence of these gradients on the diversity of the microarthropod community. In accordance with ecological theory (Grime 1979), we predict that maximal microarthropod diversity will be at intermediate levels of stress and disturbance.

Plate 3: Disturbance-stress matrix in the control plots at Sourhope

However, as noted in recent literature, below-ground diversity might neither respond to, nor share the controls that shape plant communities, so examining the response of our chosen soil fauna to these treatments will be interesting. The experiment has been running for two years and data will be available shortly.

References

Grime, J.P., 1979. Plant strategies and vegetation processes. Wiley & Sons, Chichester, England.

Lisa Cole & Richard D. Bardgett
University of Lancaster

Stirling team - micropedology meeting, Belguim

In July Donald Davidson, Patricia Bruneau and Christian Spring, all from the University of Stirling, attended the 7th International Working Meeting on Micropedology at the University of Gent in Belgium. This meeting attracted many soil scientists, micromorphologists, archaeologists, geologists and sedimentologists from all over the world to discuss the applications of micropedology to the soil environment.

At this meeting two poster and two oral presentations from Soil Biodiversity projects were given. The focus of these presentations was on the impacts of soil fauna on soil structure and how these impacts can be quantified by using soil micromorphological and image analysis techniques.

All of the work presented came from research carried out as part of the Soil Biodiversity programme at both Sourhope and the 'Sweethope' separate field sites established for our study. Both the oral and poster presentations were very well received by the conference participants and stimulated a great deal of interest, in addition to the interest created in the Soil Biodiversity Programme. Attending this conference allowed contact to be established with soil biodiversity research groups from Rennes in France and Wageningen in the Netherlands.

In addition to the above meeting, I attended the first ever Thematic School of Microscopy as Applied to Soils and Environment, which was also held at the University of Gent. This workshop was sponsored by CNRS (France) and FNRS (Flanders, Belgium) and covered a number of different microscopic and spectrographic techniques that may be applied to the soil environment. This workshop was a great opportunity for postgraduate students from all over Europe to meet and learn about cutting edge techniques and technology.

Both of these events provided excellent opportunities for members of the Soil Biodiversity Programme to highlight its existence and to spread the word of what the programme is trying to achieve, to an international audience.

Christian Spring, University of Stirling

Latest Masterclass

A masterclass entitled 'Inferring Relationships from Molecular Data' was organised for Programme members by Professor Martin Embley and his staff at the Natural History Museum, London. The event took place on 19th September 2001 and was attended by over 50 people including representatives of ten of the Soil Biodiversity awards. The meeting comprised two parts, a presentation by Professor Embley and a data workshop run by museum staff. Thanks are due to Martin Embley and the NHM for organising the event, which for many people must have been a turning point in their understanding of this ever evolving field.

More information on the course is available on the Soil Bio webpages at *www.nmw.ac.uk/soilbio/events_past.htm*
Note that a web-based course on molecular systematics and evolution is available at *www.bioinf.org/molsys*

Rebecca Pinder, CEH Merlewood

Nematode molecular barcodes

As anyone who has examined the mesofauna of Sourhope's soil will be aware, nematode worms are found in vast numbers - and, to the untrained eye, all look alike. Yet these tiny, omnipresent animals play a wide variety of roles in soil ecology - as bacteriovores, fungivores, plant browsers, plant parasites, even carnivores feeding on animals including other nematodes. However, since a million individuals may be found in a square metre of soil, and an unknown number of species exist, it is clear that novel methods are required to survey nematode diversity in a rapid and cost-effective manner.

For this reason, in Mark Blaxter's lab at the University of Edinburgh we are turning the tools of molecular biology toward the problem of characterising Sourhope's nematode community. The aim of "molecular barcoding" is to identify taxa of interest using characteristic DNA sequences, such as the small subunit (SSU) ribosomal RNA gene. The technology now exists to carry out Polymerase Chain Reaction (PCR) on a single nematode and determine its SSU sequence.

When many such sequences are compared, they can be clustered into groups of similar or identical sequences termed Molecular Operational Taxonomic Units (MOTU). Since a large SSU dataset already exists for nematodes (Blaxter et al. 1998), a newly found MOTU can either be matched to a known sequence (hence to a known nematode taxon), or, if it is novel, phylogenetic methods allow us to place it within the known tree of nematode diversity - usually allowing classification at least to family level, if not genus.

Figure 1: Light microscope image of *Helicotylenchus* sp. a plant parasitic nematode common at Sourhope

Full details of methods and preliminary survey results can be found in Floyd et al. (2002). Further samplings from Sourhope were carried out in June and October 2001. Analysis of sequences is ongoing; thus far a total of 48 MOTU have been defined from 648 sequences. It is unknown how many more are yet to be discovered.

We are also carrying out a survey of Sourhope's nematodes by traditional morphological methods, in parallel with our molecular survey. This will allow comparison of the two approaches, to determine whether they are providing approximately the same information. This will warn us of any potential biases in our molecular survey method – for example, if certain taxa are systematically missed because they fail to PCR.

Eventually, these data should allow us to develop a method of surveying nematode diversity and relative abundance directly from bulk DNA prepared from soil. Using a hybridisation approach, SSU PCR products can be arrayed on filters or microarrayed on slides and identified by DNA probes from known or indicator taxa.

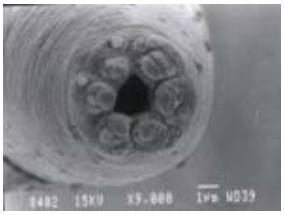

Figure 2: Scanning electron micrograph image showing the mouthparts of the bacterivore *Panagrolaimus* sp., a strain maintained in culture from Sourhope

We anticipate that this will be a powerful method for use in the analysis of nematode communities, and will lead to a greater understanding of the role of these animals in soil ecosystem function.

References

Blaxter ML, De Ley P, Garey J, et al. (1998) A molecular evolutionary framework for the phylum Nematoda. Nature, 392, 71-75.

Floyd RM, Abebe E, Papert A, Blaxter ML (2002) Molecular barcodes for soil nematode identification. Molecular Ecology, 11, 839-850.

Robin Floyd & Eyualem Abebe
University of Edinburgh

Genetic relationships in enchytraeid worms

The main objective of this small grant was to examine the diversity and functional significance of Enchytraeid species at Sourhope. Little is known about the relative importance of individual species although a typical, temperate upland grassland may hold as many as 25 species.

We hypothesised that one species, *Cognettia sphagnetorum*, would dominate Enchytraeid biomass in the control plots but anticipated that the application of lime would cause *C. sphagnetorum* to decline while other species survived. What we did not know was would this alter the functional behaviour of the different species? The key to our study was the direct linking of species identification and carbon uptake of individual worms, using a dual molecular approach of genetic marker identification and compound-specific stable isotope analyses (CSIA); most Enchytraeid worms are too small to analyse using bulk stable isotope analyses. Cholesterol was identified as the major extractable lipid component in enchytraeids and thus an ideal compound to be used as a proxy to examine assimilation rates.

Enchytraeid worms from Sourhope microcosms of C4 soil

First we developed a robust and objective taxonomy of enchytraeid species at Sourhope by integrating existing taxonomic knowledge with new approaches in molecular genetics. The DNA sequence analysis of enchytraeids revealed several distinct clades. Enchytraeid-specific polymerase chain reaction (PCR) primers were generated for each worm previously identified to genus using species morphology. Slowly evolving genes (e.g. mitochondrial cytochrome oxidase) showed little power for discerning the relationship between enchytraeid groups. More rapidly evolving genes (e.g. mitochondrial 16s rDNA) gave better resolution, with several distinct clades being observed. In most cases, each putative genus identified using classical approaches represents a monophyletic clade. The one exception is *Marionina argentea,* which falls into the *Fridericia* clade. We are currently doing more sequencing of other Marionina individuals to determine whether the *Marionina/Fredericia* complex is truly para- or polyphyletic but we can now utilise SSCP (single stranded conformational polymorphism) approaches to rapidly identify single individuals without the need for morphological identification.

The latter part of our study tested the hypothesis that the assimilation of rhizosphere carbon by enchytraeid worms could be observed through the incorporation of isotopically enriched carbon substrates, introduced to the rhizosphere via ^{13}C enriched CO_2 pulsing of the above ground vegetation (Ostle et al., 2000). Different carbon assimilation rates of individual species would correspond to differences in their functional behaviour. We found that liming reduced the uptake of carbon by Enchytraeids and that this corresponded to a reduction in carbon uptake by *Cognettia* while there was little change in carbon uptake by *Fridericia.*

Dr Helaina Black
CEH Merlewood

F&M latest from Sourhope

I would like to thank all users of the Soil Biodiversity Site at Sourhope for their patience and understanding during the Foot and Mouth Disease (FMD) outbreak in 2001. Since there have been no new cases of FMD in the UK for over seven months now, there are no longer any restrictions on visits to the site. However, it would be appreciated if all visitors could observe the following general biosecurity precautions:

- All clothing to be washed since last contact with animals susceptible to FMD
- Footwear to be disinfected using a MAFF-approved disinfectant;
- Items of protective clothing brought to Sourhope to be freshly laundered or cleaned as appropriate;
- Scientific equipment must not pose a risk of spreading disease and should be disinfected if necessary - if in doubt please refer to me prior to your visit.

Whilst at Sourhope all visitors should:

- Follow any additional advice provided by the Officer in Charge or other Sourhope staff, particularly in relation to the use of boot dips etc. upon arrival.
- Avoid all unnecessary contact with livestock and areas where livestock are handled.
- Refrain from wandering around farm buildings;
- Observe a high standard of personal hygiene;
- Ensure that all food waste is disposed of correctly.
- On completing a period of work at Sourhope it is recommended that visitors should:
- Ensure that all items of equipment and clothing are cleaned and disinfected as appropriate;
- Consider any conditions that apply regarding their next destination.

If anybody has any concerns or questions about a visit, please feel free to phone or email me.
Graham Burt-Smith, Officer in Charge, Sourhope,
Tel 01573 420585
Email g.burt-smith@macaulay.ac.uk

Staff Changes in Programme Office

Richard Scott retired from CEH Merlewood in November 2001. Richard was the Programme Manager for the Soil Biodiversity Programme since its start in 1997. He was instrumental in helping to establish the field site at Sourhope, and he has played a key role in the administration of the Programme. We wish him a happy and fulfilling retirement. The Programme will now be jointly managed from CEH Merlewood by myself and Professor Mike Hornung, assisted by Rebecca Pinder who has returned to Merlewood after a long illness.

Lynne Irvine has been appointed Data Manager, and she will also be based at CEH Merlewood.

Andrew Sier, CEH Merlewood

Stirling Meeting

Progress Review Meeting, University of Stirling

A common feature of the Programme's annual meetings for award holders is, it seems, atrocious weather, and the meeting in January 2002 was no exception. Delegates battled against hurricane-force winds, with gusts in excess of 80 miles an hour, to reach Stirling, despite widespread disruption to trains, planes and road traffic.

A full programme of talks was opened Allan Wilson, MSP Scotland's Deputy Minister for Environment and Rural Development, who explained some of the soils-related policy initiatives in Scotland. Delegates then heard progress reports from each of the Phase I awards, as well as an overview of planned Phase II work. Potential applications of the work were discussed, as well as future directions for soil biodiversity and ecosystem function research.

Prizes were awarded by Sheila Anderson, Head of NERC's Communications Directorate, to the presenters of the best poster and paper .Winners were Caroline Fenwick for best paper by a PhD student, Mark Bradshaw for best paper by a PDRA and Lisa Cole for best poster. Congratulations to them all!
Thankfully by the end of the meeting, the foul weather had eased, hopefully enabling people to enjoy a less eventful journey home. The Steering Committee met immediately following the main meeting, and dealt with a range of issues relating to the Programme, including plans for a modelling component, widely viewed as an essential next step.
The Programme Office are grateful to Donald Davidson and staff at the University of Stirling, and also to Christine Donn at CEH Merlewood, for organising an excellent meeting. Thanks also to everyone who demonstrated their dedication to the Programme (or perhaps their foolhardiness!), in reaching the meeting despite the weather.

Andrew Sier, CEH Merlewood

DATES FOR THE DIARY

Soil Biodiversity Steering Committee
MRC, London - 19th June 2002
British Society of Soil Science
Soils as carbon sinks : opportunities & limitations
Scientific Societies Lecture Theatre, Central London
28th June 2002
British Society of Soil Science conference
Seale-Hayne Campus, University of Plymouth
8 -11th September 2002
BES Symposium on Soil Biodiversity & Soil Biodiversity Annual Meeting
University of Lancaster - 25 to 28 March 2003

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