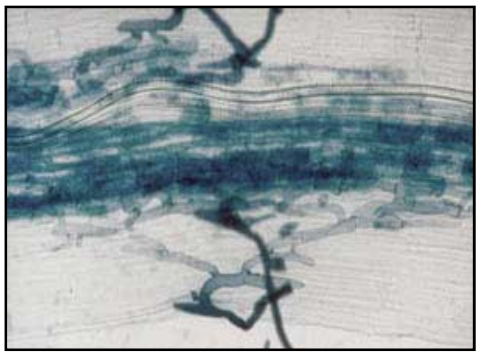
Sampling and counting soil fauna

Follow-up notes from workshop on soil biology held with University of Western Australia and Bugs & Biology Grower Group

Friday 20th July 2012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The information presented in these notes was prepared by the SPICE program for the 'Monitoring Soil Science' project. SPICE is a secondary science teachers' enrichment program and is a partnership between the WA Department of Education and The University of Western Australia. For more information: www.spice.wa.edu.au

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These notes give an overview of the methods used to extract and count soil fauna, as demonstrated by staff and students from the University of Western Australia during the workshop co-ordinated by Bugs & Biology Grower Group on Friday 20 July. The notes include the following components:

1) Equipment
2) Safety aspects
3) Collecting soil samples
4) Extracting soil fauna
5) Identifying and counting soil fauna
6) Further information
7) Background sheet on mites and springtails

We are grateful to the SPICE program for permission to reproduce extracts from their manual on 'Monitoring Soil Science' (http://soils.duit.uwa.edu.au/index.php)

1. Equipment

To **sample the soils** you will need:

- Sampling ring (e.g. cut of section of PVC pipe. Cut to 6 cm lengths and mark a few dots or crosses at 1 cm depth inside the ring with permanent marker)
- Trowel
- Flat surface large enough to cover surface of sampling ring (e.g. paint scraper)
- Plastic zip-loc bags
- Gloves

To **extract soil fauna** you will need:

- Soil samples
- Funnel and container (made from 1.5 L soft drink bottle)
- Lamp (source of heat and light)
- Gauze swabs (2 per sampling apparatus)
- Mesh (square of flywire)
- Collecting containers with lids of approx 70 mL (e.g. specimen containers), preferably with 1 cm layer of plaster of paris and charcoal at the bottom (see instructions)

To **identify soil fauna** you will need

- Classification sheets (to note down results)
- Basic identification charts (e.g. http://soils.duit.uwa.edu.au/students/instructions.php)
- Microscope
- Container with collected soil samples
- Petri dish or other clear, flat dish
- 70% alcohol to preserve specimens
- Specimen tube if you wish to keep specimens

2. Safety aspects

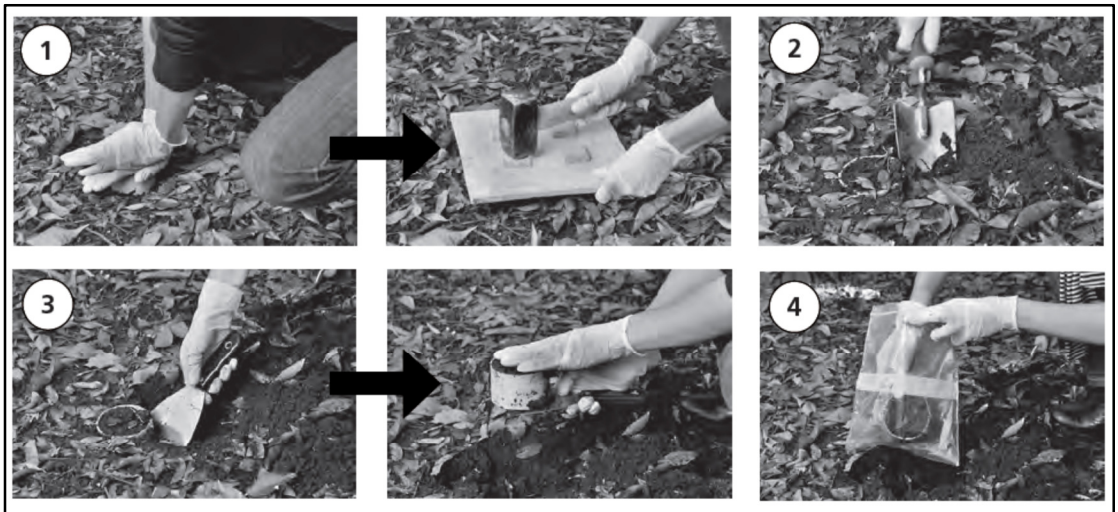
SAFETY

Handling soil and compost: Always wear safety glasses. Do not ingest or smell the soil/compost, and avoid inhaling its dust, since harmful bacteria and fungi could be present. Wash your hands thoroughly with soap and water afterwards.

Handling alcohol: Always wear safety glasses and avoid contact with skin. Keep alcohol away from heat, sparks or open flames. Avoid breathing in vapours and ensure the room is well ventilated.

3. Collecting soil samples

- We recommend soil samples are taken first thing in the morning. The ground is cooler then, and soil fauna are likely to be nearer the surface.
- Soil fauna abundance will vary throughout the year. Soil fauna biomass tends to be greatest in the top few centimetres of soil when the soil is moist. In dry summer months soil fauna abundance made be lower, or you may find that the species that are present are very different to those present in winter.
- When sampling soils, record the date, time, ambient temperature (approx) and weather conditions (sunny, raining, overcast).
- Push the sampling ring 5 cm into the ground. Where a litter layer is present, you should also collect whatever litter is found in the top 1 cm of the sampling ring (which should be above the level of the soil). If the litter layer is greater than 1 cm, brush off the top so that only the lower 1 cm is collected.
- See below for step-by-step instructions on sampling soil.



How to sample...

1. Push your sampling ring into the soil. If the ground is hard you can tap the ring into the soil by placing a wooden board over top, and hitting it lightly with a mallet.
2. Dig around one side of the ring with a trowel.
3. Slide the scraper straight underneath the ring. This stops you losing soil when you lift the sample out.
4. Place the sample into a plastic bag. Seal to ensure nothing can escape, then write on its sample number and place in an esky.
5. Repeat for all the samples and take them back to the lab for processing.

If you wish to keep your soil fauna alive, follow the instructions below to prepare a layer of permeable material at the bottom of the collecting jars *prior* to taking soil samples. Periodically add a couple of drops of water to the containers to keep the organisms alive and moist during the extraction.

Before the sampling takes place prepare collecting containers with a mixture of plaster of Paris and charcoal. This base will help keep any soil fauna alive during the extractions.

Plaster of Paris and charcoal recipe

- **8 parts plaster of Paris (approximately a tablespoon per container)**
- **4 parts activated charcoal**
- **6 parts water (enough to mix to a soupy consistency)**

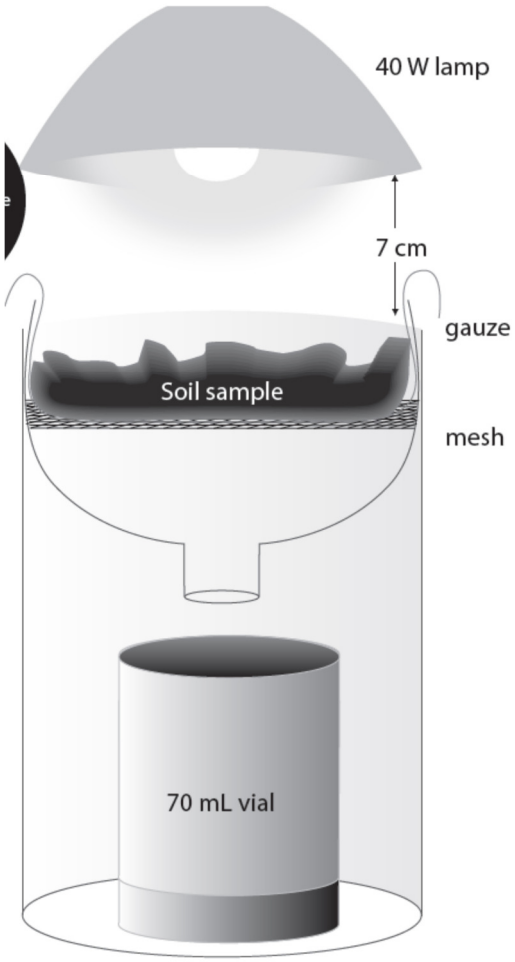
Mix the dry plaster of Paris and charcoal together, then sieve into water. Allow to stand for 5 minutes before stirring into a thick, soupy consistency. Pour into a container to a depth of 1cm. Smooth by tapping on a workbench and swirling. Leave to dry for about a couple of days. When you re-wet the plaster of Paris it helps to tilt the container and drop the water on from one side, this stops trapped airbubbles lifting the paster from the base of the container. Pour off any excess water.

4. Extracting soil fauna

A simple device for extracting soil fauna can be made by cutting the top of a 1.5 L soft drink container, and using this as a funnel to collect soil fauna in the bottom of the soft drink bottle. The instructions below detail how to make this simple device; these are the same instructions that were used to create the sampling devices on display during the field day.

Instructions:

1. Place the collecting container (lid off) inside the plastic beaker, and rest the funnel over top (see diagram). The collecting container's damp plaster of Paris base will help keep your soil fauna alive.
2. Place one layer of mesh and two gauze swabs inside the funnel. These prevent chunks of soil falling into your collecting jar, yet still allow the soil fauna through as they flee the lamp's heat.
3. Empty your soil sample into the funnel, shaking any loose bits of soil from the bag. Label the apparatus so you know which sample is in the funnel.
4. Finally, position a lamp approximately 7 cm above the funnel.
5. Check the equipment daily for the next 3 to 5 days. As the sample dries out you should start to see tiny soil animals in the collecting container underneath. Make sure the plaster of Paris base stays slightly damp (but not soaked), otherwise your soil animals will dry out or drown!



You can put sand or gravel in the bottom of the 1.5 litre soft drink container to stop the bottle from accidentally tipping over.

You should leave all your soil samples for around the same time (e.g. all 48 hours regardless of when collected)

5. Identifying and counting soil fauna

- Look at the soil fauna under a microscope. Use the mite and springtail identification sheets to count the number of mites and number of springtails in each sample. Record your results on your record sheet.
- If the mites and springtails are too mobile to count, tip them into a Petri dish containing a thin film of 70% alcohol (if there is too much liquid, the animals will move around as you move the dish, making it difficult to keep track of how many animals there are.
- You may like to keep your specimens afterwards by preserving in 70% alcohol and labelling.
- You can compare soil fauna abundance and diversity from different parts of your property, or you might like to compare soil from one part of your property at different points in time. Soil fauna are known to be highly variable in space and time, so it is advisable to take multiple samples (perhaps 3 to 5) from a particular area of interest to avoid an 'atypical' result.
- It is difficult to specify an 'ideal' soil fauna result or community for any particular area, given that soil fauna are so diverse and occupy many ecological niches. Generally speaking, a 'healthy' soil fauna community is diverse (i.e., many different types of soil fauna are present, without overdomination by one species) and abundant (i.e., relatively high number of individuals).

6. Further information

Fact sheets with identification pictures are available on springtails, mites and soil fauna: http://soils.duit.uwa.edu.au/students/instructions.php (available as .pdf files)

Podcasts demonstrating the methods outlined in this note:
- Sampling soil: http://soils.duit.uwa.edu.au/mediafiles/soilsampling/index.php
- Extracting soil fauna: http://soils.duit.uwa.edu.au/mediafiles/extractingsoilfauna/index.php

Further information on the Monitoring Soil Science project with schools is available at: http://soils.duit.uwa.edu.au/index.php

For further detailed information on soil biology, see 'Soils are Alive' on: http://www.soilhealth.com/soils-are-alive/

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7. Background sheet on mites and springtails

The most abundant soil dwelling microarthropods are springtails and mites.

Springtails (Collembola)

Springtails are primitive, wingless microarthropods, closely related to insects [1]. They're among the oldest of terrestrial animals, with a 400 million year old Collembola fossil discovered in Scotland.

The name 'springtail' derives from a lever-like hinged spring, the furcula, located on the underside of their abdomen. This appendage is held under tension, and once released, snaps against the ground propelling the springtail up to several times its own body length. In the tiny, dark air spaces between soil particles such an adaptation is of limited use, so many soil dwelling species have a reduced (or absent) furcula.

Similarly, you may see black, light-sensitive pigment spots on the heads of some springtails, but not on those that dwell deep in soil or in caves.

All springtails possess six legs and a pair of antennae, and they tend to fall into one of three body types: a bulbous, rounded form; an elongate form with pronounced furcula; and an elongate, soil dwelling form, with reduced antennae and furcula.

Springtails are found throughout the world from the Antarctic to the Himalayas with new species still being discovered. Over 7800 species are currently listed worldwide, with about two thousand species in Australia [1].

Their habitats range from the soil to leaf litter, bark to caves and even the surface of stagnant ponds. Most springtails are detritivores, feeding on dead organic matter after it has been colonised by microbes, although some are fungivores while others graze on algae and lichen. Their feeding helps break up the leaf litter, increasing its surface area and introducing bacterial cells and fungal spores to new areas. Only a few springtail species are pests, such as the introduced lucerne 'flea' that can damage Australian crops. In turn, springtails are a major food source for a variety of soil predators, including ants, mites and pseudoscorpions.

Springtail numbers tend to be highest where there's a combination of warmth, moisture and high organic matter content. Desiccation is potentially lethal since most springtails respire directly through a porous cuticle.

A single habitat may support 20 or more species [2]. Under ideal conditions populations can increase considerably, forming large aggregations. Population densities in the soil and litter have been measured at over 500 000 springtails m^{-2} although in Australia densities tend to range from 2000 to 30 000 springtails m^{-2} [1].

Larvae and a succession of immature stages all closely resemble the adults. Individuals moult as they increase in body size. Adult springtails continue to moult, although with minimal changes in size. Some species may moult up to 50 times over their life span.

Large springtails are visible to the naked eye, and most species fall in the range of 0.2 to 3 mm (one or two are even larger, such as a Central European species that reaches 9 mm in length). To help students estimate the insect's size, try placing a pin underneath the dissecting microscope for comparison.

Mites (Acari)

Mites, like spiders, belong to the class Arachnida, and at first glance, both are visually similar. However, mites are distinguished from spiders by their lack of a waist between the thorax (where their legs are attached) and abdomen. This characteristic gives the appearance of a single oval body.

A typical life cycle of a mite includes egg, larva, nymph (protonymph, deuteronymph, tritonymph) and adult, although there's often variation between species. Individuals possess eight legs in their adult and nymph stages, but only six as larvae. Like springtails, mites been around for a long time, with fossils dating back nearly 400 million years [3].

Mites don't possess antennae and have no recognizable head, although their front end should have visible pincer-like mouthparts. Most measure less than 1 mm, although with a size range of 0.1-3 mm some species are just visible to the naked eye. Certain species appear hairy, and come in a wide range of colours.

Acari is a very diverse order, that includes parasitic species as well as free-living, and they've colonised nearly every known terrestrial, marine and freshwater habitat. Most terrestrial mites live in the soil and leaf litter, although some are also found in plants, mosses and lichens. They include some major economic pests such as spider mites.

About 48 000 species of mite have so far been described, but this is likely to be a fraction of all species [4]. In all but the driest of environments there can be hundreds of thousands of mites per square metre of soil [5].

Soil mites include slow-moving fungivores and detritivores, as well as fast, long-legged predators that hunt small invertebrates such as springtails, other mites and nematodes. Like spiders, predatory mites can inject a digestive liquid into their prey.

Grazing by mites helps to regulate microbial activity, and in some cases their feeding has even been shown to stimulate microbial activity, just as sheep grazing on grass stimulates new pasture growth[6]. Experiments that have removed mites from a substrate demonstrated that the organic matter decomposition rate slows by an average of 23% [6].

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