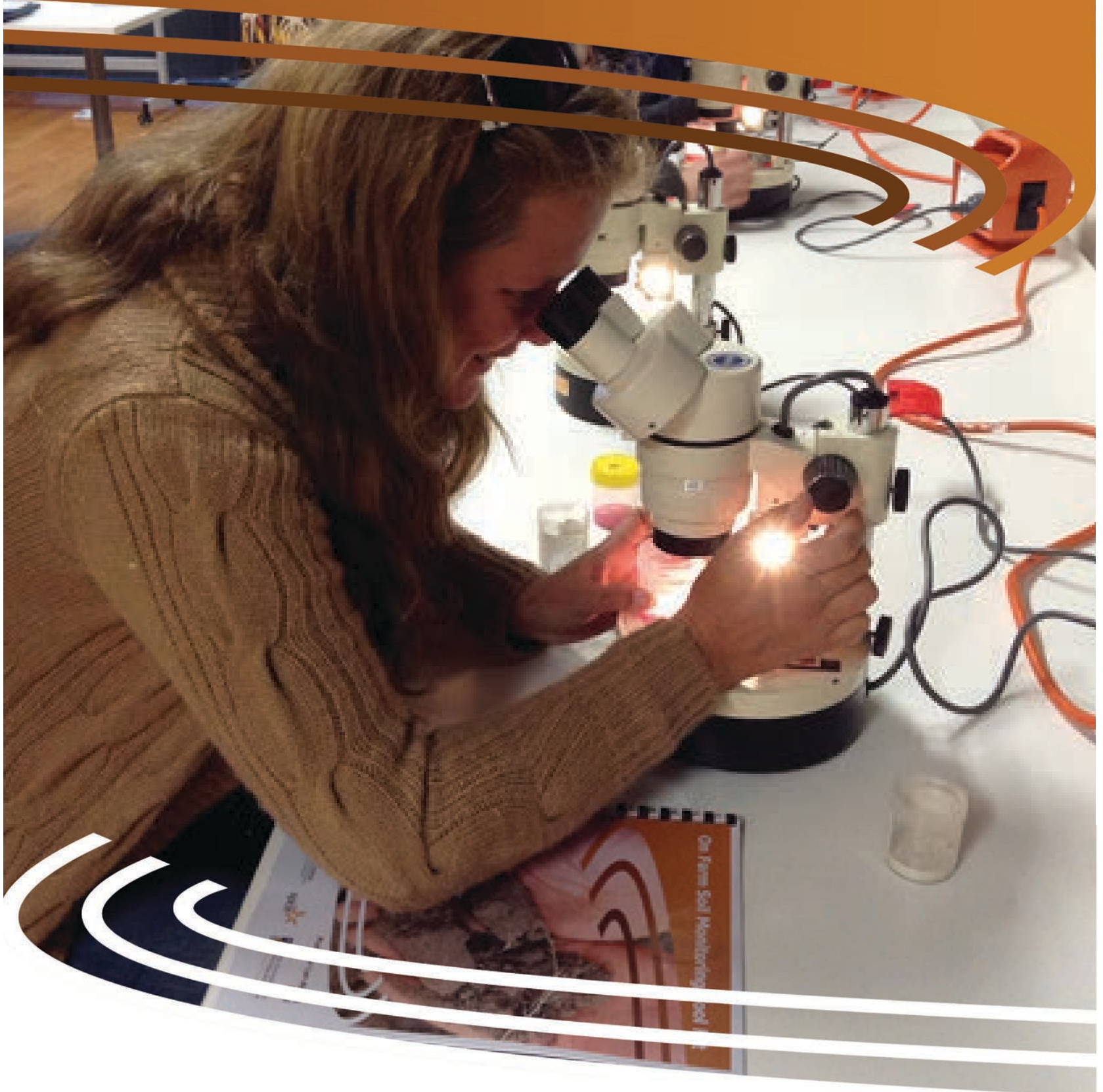


On-Farm Soil Monitoring Handbook





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Overview

The On-Farm Soil Monitoring project is a joint project led by Wheatbelt NRM, the South West Catchments Council and The University of Western Australia. It is designed to introduce landowners and farmers to the diversity of organisms in their soils and to the use on-farm soil monitoring methods. This On-Farm Soil Monitoring Handbook was developed as part of this project and explains procedures for assessing soil fauna (especially mites and springtails) and the mycorrhizal fungi inside roots of crop and pasture plants as well as many Australian plant species. Soil mites and springtails both have important roles in chemical, physical and biological components of soil health. They are sensitive to land management practices and can be assessed on-farm with relatively simple methods and equipment.

This On-Farm Soil Monitoring Handbook includes information about how to sample soil and roots and how to extract and count the dominant soil mesofauna (mites and springtails). It also offers suggestions for on-farm research activities such as investigating the effects of land management practices on the abundance and diversity of soil fauna and on the presence of mycorrhizal fungi inside roots. By following the soil monitoring suggestions in this Handbook, farmers have an opportunity to increase understanding of factors that influence the health of their soils.

On-farm monitoring of soil fauna and mycorrhizal fungi contributes local knowledge that is relevant to how agricultural management practices influence soil conditions within and between seasons. It focuses on soil biological fertility as well as on soil chemical and physical fertility.



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1.0 Background

This On-Farm Soil Monitoring Handbook was developed because of the increasing interest in Soil Biology within the Wheatbelt community.

The On-Farm Soil Monitoring Handbook is based on the 'Monitoring Soil Science' resource developed by SPICE, a collaborative program between the Western Australian Department of Education and The University of Western Australia. The SPICE 'Monitoring Soil Science' resource helps teachers introduce scientific methodologies, including soil sampling strategies, to students and provides a platform for ongoing soil-based scientific research. The On-Farm Soil Monitoring Handbook has adapted this resource for use by farmers as a tool for monitoring aspects of soil health.

The On-Farm Soil Monitoring Handbook is used in conjunction with practical hands-on workshops that demonstrate the procedures for assessing soil fauna and mycorrhizal fungi.

2.0 Soil

Soil is a very valuable farm resource. It is a complex and fragile medium in which there are interactions between water, air, minerals, organic matter and roots. Soils contain pore spaces with varying proportions of air and water. Soil is made up of weathered parent bedrock and deposited minerals.

Organic matter is a relatively small part of the soil, encompassing plant and animal material both living and dead, but its influence is vast. Organic matter is crucial to soil's chemical fertility, resilience and structure, and it is the source of energy and carbon for many soil organisms.

2.1 Diversity of soil organisms

Soil organisms include bacteria, fungi, microarthropods, nematodes, earthworms and insects. Most soil organisms are largely dependent on soil organic matter or living plants and perform a number of vital processes in soil. Some of them are involved in the transformation of inorganic molecules in soil and are not directly involved in the soil carbon cycle, but they are indirectly influenced by organisms that cycle soil carbon. While certain soil organisms (such as plant pathogenic nematodes) may have detrimental effects when they are present in high numbers, in a healthy soil, they rarely become pests.

Soil organisms individually or in combination have roles including:

- I. helping soil to form from original parent rock material,
- II. contributing to the aggregation of soil particles,
- III. enhancing cycling of nutrients,
- IV. transforming nutrients from one form to another,
- V. assisting plants to obtain nutrients from soil,
- VI. degrading toxic substances in soil,
- VII. causing disease in plants,
- VIII. minimizing disease in plants, and
- IX. assisting or hindering water penetration into soil.

[See www.soilhealth.com for more information]

The diversity of soil organisms is far greater than the diversity of plant communities anywhere on Earth. A square metre of soil contains millions of bacteria, kilometres of fungal hyphae and thousands of mites and springtails. When organic matter is incorporated into soil a chain of interactions involving soil organisms is initiated, and nutrients are cycled during this process.



Figure 1 Dung beetles incorporate organic matter into soil and this stimulates the activity of other soil organisms, including soil mesofauna. (Photo: L Abbott)

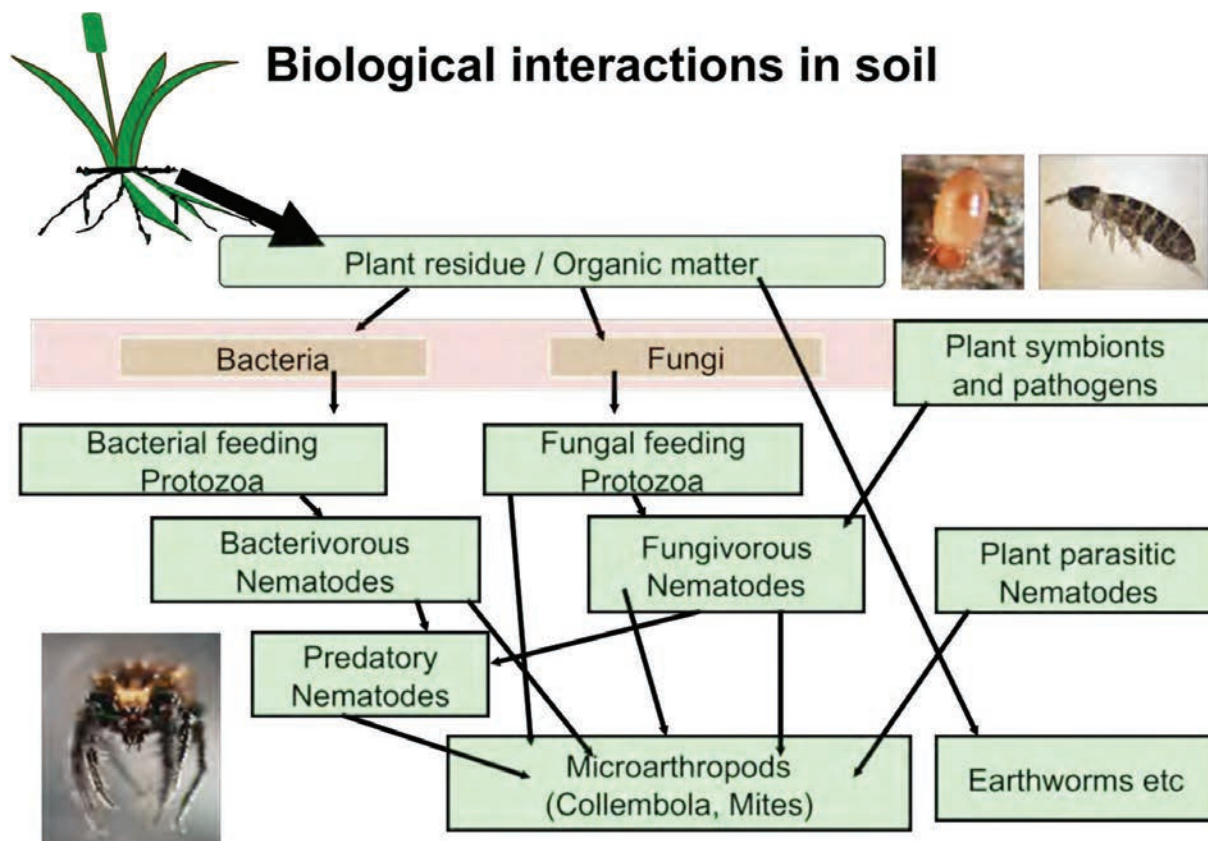


Figure 2 Organic matter in soil is the trigger for a sequence of interactions between groups of soil organisms involved in the cycling of nutrients from dead organic matter (Based on a poster by P van Vliet and VVSR Gupta)

2.2 Soil fauna

Soil fauna play a role in the breakdown (decomposition) of organic matter. The most common mesofauna are mites and springtails which are about 0.2 to 0.5 mm in length.



Figure 3 Mites and springtails are common soil mesofauna and occur in all soils (Photos: B. Mickan)

2.3 Mycorrhizal fungi

Almost all plant species form mycorrhizal associations. Mycorrhiza means 'fungus root' and refers to several kinds of specialised associations between fungi and roots. The mycorrhizas associated with most agricultural plants are called arbuscular mycorrhizal (AM) fungi. These associations are generally beneficial to both the plant and the fungus. Nutrient exchange occurs between the partners and there are also other benefits such as improvement in soil structure and enhancing access to moisture in dry soil. AM fungi are dependent on the host plants for carbon. They cannot grow independently in soil. Under some conditions, there is no beneficial effect from the presence of AM fungi in roots, and plant growth may even be reduced.

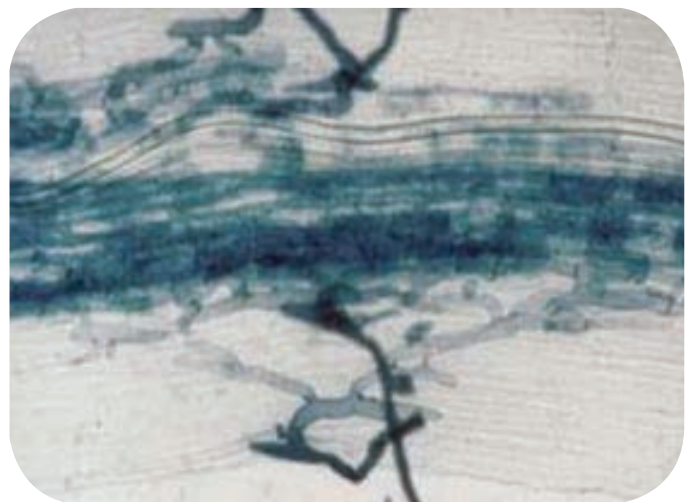
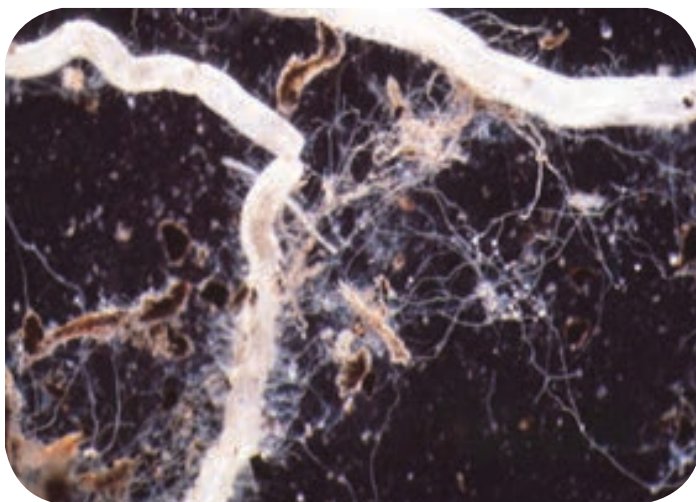


Figure 4 Mycorrhizal subterranean clover roots (left: unstained, with fine strands of fungus (called hyphae) outside the roots) and mycorrhizal subterranean clover roots (right: stained roots) demonstrating the presence of the fungi inside the cells and intercellular spaces of the root. (Photos: L Abbott)

3.0 Sampling soil and roots

When sampling soil for Soil Fauna Analysis, you need to take several samples from a defined area of approximately 4m x 4m. You should only sample the top 5cm of the soil. Each plot needs to be easily identified so they can be re-sampled later so that any changes can be monitored. It is best to take several randomly placed samples within the plot. It is a good idea to collect the soil samples in the morning and then keep them in a cool place (i.e. not in the sun).

3.1 Soil sampling equipment

For soil sampling you need: (i) a PVC sample ring (5cm depth), (ii) a trowel or spade, (iii) plastic sandwich bags and (iv) a permanent marker.

See the SPICE podcast for soil sampling at

<http://soils.duit.uwa.edu.au/mediafiles/soilsampling/index.php>



Figure 5 When taking a soil sample for extracting soil mesofauna, the same volume of soil needs to be collected for each replicate sample (Photo: SPICE, UWA)

3.2 How to collect soil samples

1. Push the PVC sampling ring into the soil until its top lip is 1 cm above the soil surface.

If the ground is hard, tap the ring into the soil by placing a wooden board over the ring, and hit it lightly with a mallet. If there is a thick layer of leaf litter, brush it off so that it is level with the top of the ring.

2. Dig around one side of the ring with a trowel.

3. Slide the scraper straight underneath the ring to prevent the soil falling out of the ring.

4. Place each soil sample into a separate plastic bag. Seal and label with a permanent marker. Keep them in a cool place – out of the sun.

3.3 How to collect root samples (for mycorrhizas)

Gently dig around a plant with a trowel and loosen the soil. Shake the roots to remove most of the soil, place in a plastic bag and label. Keep in a cool place out of the sun.

3.3 How to collect root samples (for mycorrhizas)

Gently dig around a plant with a trowel and loosen the soil. Shake the roots to remove most of the soil, place in a plastic bag and label. Keep in a cool place out of the sun.

4.0 Extracting Soil Fauna

Before setting up the soil fauna extracting apparatus you need to prepare specimen containers with layer (up to 1cm) of plaster of Paris. It is a good idea to add powdered charcoal or similar material to the plaster of Paris to give it a darker colour. This solid base within the sampling jar can moistened during the extraction of the soil fauna.

4.1 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 and slowly add the water.

Allow to stand for 5 minutes before stirring into a thick, soupy consistency.

Make sure it forms a smooth paste.

Pour into a sampling container to a depth of about 1 cm. Smooth by gently tapping on a workbench. Leave to dry for at least a day before using.

It is important to slightly moisten the plaster of Paris in the sampling jar before collecting the animals to help avoid the soil fauna dying during their extraction from soil.

4.2 Setting up the soil fauna extracting apparatus (Tullgren Funnel)

There are two ways to extract the soil fauna from the soil. Either use a Light Box (with several Tullgren Funnels) or make your own apparatus out of a 1.5L plastic soft drink bottle. Both methods work in the same way. The light is positioned above the soil to encourage the soil fauna to move away from the heat and light. The soil fauna migrate down through the gauze, to the sieve and down the funnel into the specimen jar. Check the fauna extraction regularly over the first few hours. Make sure the plaster of Paris remains moist, but not saturated.

The number of soil fauna in your soil samples might be low. Use a digital dissecting microscope (20 to 40 x magnification) to compare your soil fauna with those on the fact sheets.

See the SPICE podcast for extracting soil fauna

<http://soils.duit.uwa.edu.au/mediafiles/extractingsoilfauna/index.php>

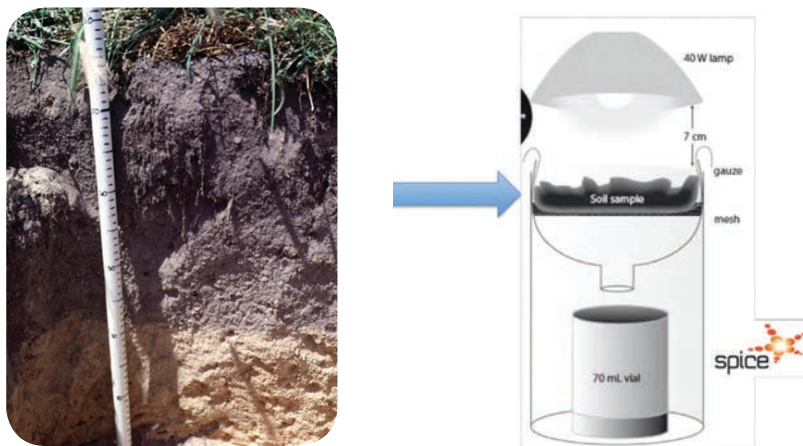


Figure 6 Taking a soil sample for extracting soil mesofauna using a Tullgren Funnel system. These organisms are usually most abundant in the upper layers of the soil profile (Photos: (left) L Abbott; (right) SPICE, UWA).

WARNING: There is always a danger in leaving an incandescent globe switched on in an unoccupied area. Make sure the lamp has metal rather than plastic fittings. Switch the light off when it is not attended at the end of each day, and run the experiment for more than one day if necessary. Take care when dealing with soil. Avoid breathing dust.

4.3 What you need when extracting soil fauna using a Light Box

- 2 x soil samples per plot (at least)
- 2 x funnels
- 2 x PVC pipe sieve rings
- 4 x gauze swabs
- 2 x 70 mL collecting containers, with 1 cm plaster of Paris containing charcoal (for colouring) in the base.

When using a soil Light Box you can test several samples at one time.

Each sample requires its own funnel, sieve, 2x gauze and collecting container.



Figure 7 Tullgren Funnel apparatus for extracting soil fauna in a Light Box. The Men's Shed in Northam, WA built these 'light boxes' for use by farmers within the Wheatbelt NRM region (Photo: L Abbott; Leibe Group Workshop).

1. Place the collecting container (lid off) on the lower level under the hole, inside the Light Box and rest the funnel over top.
2. Place one of the aluminium sieves and two gauze swabs on top of the funnel. These will prevent soil falling into your collecting jar but allow the small soil fauna to fall through.
3. Empty one soil sample into each sieve and place in the funnel. Label the sample.
4. Close the door of the Light Box and turn the lights on.
5. After approximately 2 hours you can check the extraction process.. 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).



Figure 8 Examining soil fauna extracted from soil at the Leibe Group Workshop (Photo: L Abbott).

4.4 What you need when extracting soil fauna using a home-made apparatus

Make your own extracting container by cutting the top off a plastic drink bottle (1.5 L). Use the top of the bottle as a funnel. The collecting container is placed inside the bottle. It is recommended that you place some soil in the bottom of the bottle so the collecting container sits firmly. When using the home-made apparatus, you will test one sample at a time. You can either make more than one extracting jar, or complete one sample then run the next sample.

Follow the instructions above (section 4.3) for extracting the soil fauna.

4.5 Soil fauna fact sheet (SPICE Soil Science Resource)

fact sheet

Soil fauna

Here are some animals you might see under the microscope...

Magnification x 10

ant

pseudoscorpion

termite

hopper

nite

springtail

nematode

unsegmented, transparent

Smaller still

The soil is alive with microscopic bacteria, fungi and protozoa, but you'd need a really powerful microscope to see them. One teaspoon of soil can contain up to 1 billion bacteria!

Images
Honorary Associate Professor Adriano Klueber

spice

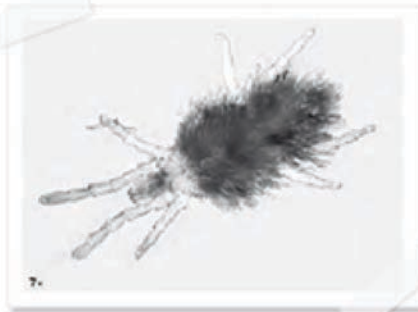
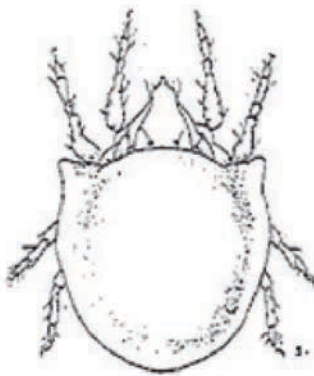
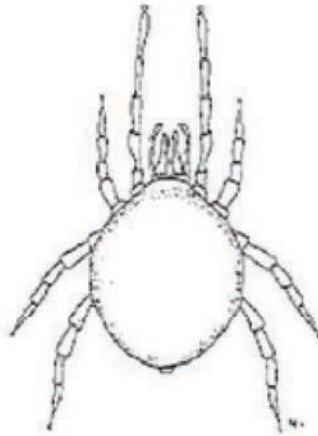
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Soil fauna (fact sheet) | page 2

4.6 Mite fact sheet (SPICE Soil Science Resource)

fact sheet

Mites

These are all mites



Images
1-3, 6-7: Honorary Associate Professor Adrienne Kinner
4-5: H Pauline McCall (1981) DSIIR

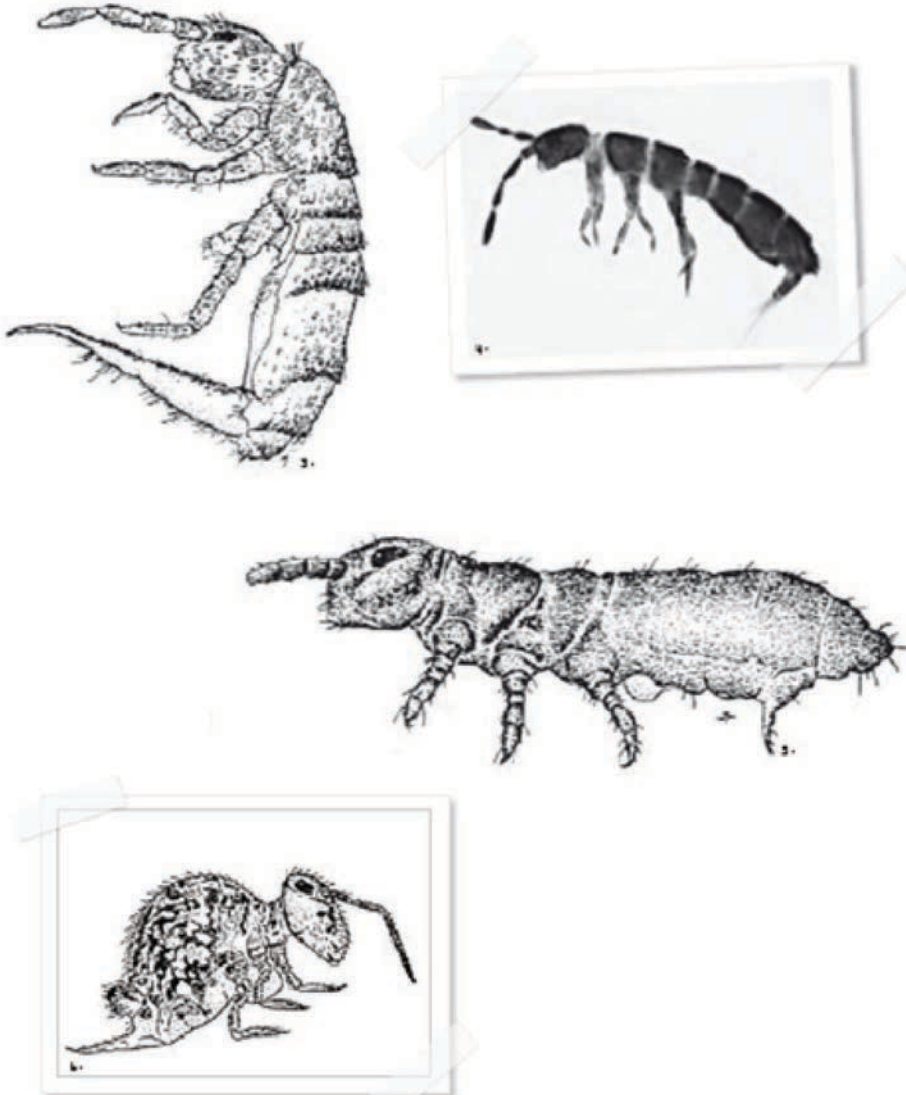
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Mites (fact sheet) | page 2

4.7 Springtail fact sheet (SPICE Soil Science Resource)

fact sheet

Springtails

These are all springtails



Images
1, 4. Honorary Associate Professor Adrienne Kinnear.
2, 14. Pauline McCall (1981) DSE.
3, 5-6. Jennifer Thuremer, South Australian Museum

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Springtail fact sheet / page 2

5.0 Staining roots for mycorrhizal fungi

Collect root samples from a paddock or from 'bioassay pots' (see below) and wash gently under a tap to remove most of the soil particles. Take a small root sample (several grams) and treat in the following way to stain the fungi inside the roots.

As an alternative to staining roots collected directly from the field, you may set up 'bioassay pots' by collecting soil (top 10 cm), placing soil in nursery pots, sowing with seeds (e.g. wheat or clover) and watering until grown for 5 to 6 weeks.

Materials required for staining mycorrhizal fungi

- Small flat plastic or glass dishes
- Forceps
- Small glass/plastics tubes with caps
- Potassium hydroxide (KOH)
- Vinegar
- Black Ink
- Light microscope (compound)
- Plant roots

Staining Solutions (Safety Advice: Avoid skin contact with KOH)

Prepare the following solutions

10 % KOH (10 g KOH in 100 ml water)

5 % vinegar (5 mL vinegar + 95 mL water)

5 % black ink (5 mL black ink + 95 mL of 5% vinegar)

Staining Method (Safety Advice: Avoid skin contact with KOH)

See the following reference for more information:

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* 64: 5004–5007

1. Gently remove the plant from the soil
2. Clean the roots in tap water and cut roots into small pieces (1-2 cm)
3. Put each root sample in small glass/plastic tubes and cover well with 10 % KOH
4. Keep roots in 10% KOH for 5-7 days at room temperature or boil for 10-30min. If the solution becomes dark brown, replace with fresh 10% KOH solution)
5. Gently rinse roots several times with tap water
6. Gently rinse roots with 5 % vinegar once
7. Add 5 % black ink and leave overnight or boil for 1-5min
8. Gently rinse with tap water once
9. Place root onto a flat dishes and cover with water
10. View under a dissecting light microscope.
11. Stained root samples can be stored at room temperature for several weeks

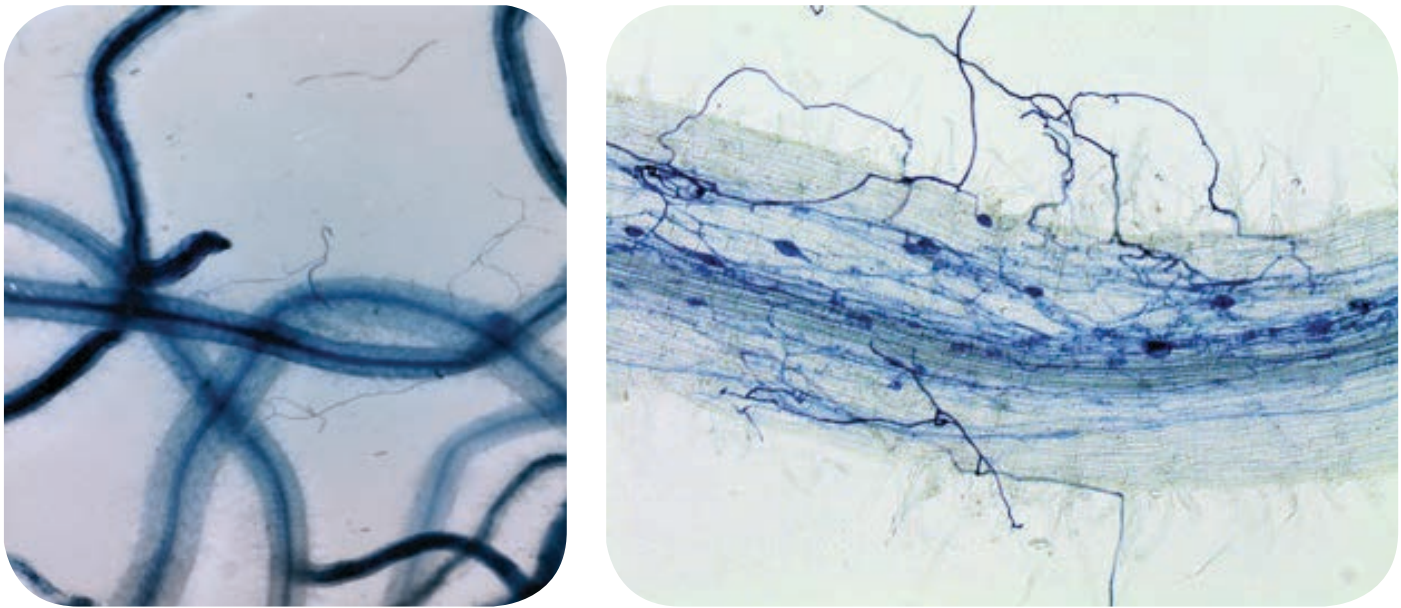


Figure 9 Roots stained to show mycorrhizal roots examined using a dissecting microscope at 20 or 40 x magnification (left). For more detailed examination (right), 200x magnification is preferable but it is not essential for assessing the proportion of roots colonised (Photos: (left) L Abbott; (right) B Mickan)

6.0 Design your own on-farm soil monitoring investigation

6.1 What do you want to investigate and monitor?

- Differences between paddocks with different management practices?
- Differences between different times during the year?
- After different amounts of rainfall?
- At different depths of soil?

Sites selected	Land Use	Your question....
Example 1	2012: Sheep & Pasture 2013: Wheat 2014: planned use	
Example 2	2012: Wheat 2013: Sheep & Pasture 2014: planned use	

6.2 Setting up your investigation

When monitoring each site you will need to set up a table to record your results as shown in the examples below:

Example 1: Sample Site Location Identification:

White Dam Paddock / GPS coordinates

Current Land use	Date sampled	Date fauna count	Mites (Number)	Springtails (Number)	Ratio Mites : Springtails
Paddock in crop (wheat)					
Paddock in crop (wheat)					

Example 2: Sample Site Location Identification:

Red Dam Paddock / GPS coordinates

Current Land use	Date sampled	Date fauna count	Mites (Number)	Springtails (Number)	Ratio Mites : Springtails
Pasture					
Pasture					

6.3 Example of data sheet – Case study at UWA's Future Farm near Pingelly, WA

The following data for soil fauna and mycorrhizas (sites A to H) were collected from 8 paddocks on the UWA Future Farm at Pingelly, WA by Cameron Gardner who is a student at Shenton College in Perth. Cameron counted number of mites and springtails for each of the soil samples and used this to estimate the number per square metre.

Table 1. Number of mites and springtails, ratio of mites and springtails, mycorrhizas (proportion (%) of root length colonised by arbuscular mycorrhizal fungi) and soil P (bicarbonate extractable) for soil samples from 8 sites with different land use histories on the UWA Future Farm at Pingelly, WA.

Land Use	Site	Mites (Number per m ²)	Springtails (Number per m ²)	Ratio Mites: Springtails	Mycorrhizal colonisation of roots (%)	Soil P $\mu\text{g/g}$
A Grazing / Stubble	 	2.2×10^3	21.1×10^3	1 : 9.6	27	55.1
B Grazing/ Stubble	 	3.9×10^3	30.0×10^3	1 : 7.7	33	28.4
C Recently Ploughed	 	0.6×10^3	22.8×10^3	1 : 38	7	61.1
D Cropped previous year	 	3.3×10^3	12.8×10^3	1 : 3.9	12	27.0
E Undisturbed bush land	 	8.9×10^3	2.2×10^3	1 : 0.2	38	24.3
F Grassed bush land	 	4.4×10^3	6.7×10^3	1 : 1.5	38	19.2
G Stubble/ ploughed	 	4.4×10^3	5.0×10^3	1 : 13	32	36.1
H Stubble/ pasture	 	3.9×10^3	1.7×10^3	1 : 0.4	18	35.4

The 8 sites sampled included a range of land use histories: (i) pasture with stubble remaining from a previous crop which was at least 2 years old, (ii) one year old stubble currently grazed by sheep, (iii) recently ploughed soil prior to cropping, and (iv) undisturbed bush land sites.

In this preliminary monitoring, there was a range in abundance of mites, springtails and mycorrhizal fungi. Mites were most abundant in soil from undisturbed bush land (Site E) where there were no understory plants. Mites were least abundant in the recently ploughed soil (Site C). Springtails were most abundant at the pasture site after crop (Site B) but least abundant at another site with a similar history (Site H). The highest ratio of mites to springtails (1:38) occurred at Site C (recently ploughed) and the lowest ratio of mites to springtails was recorded at Site E (undisturbed bushland) and Site H (pasture with stubble from previous crop).

The two sites with the highest proportion of roots colonised by mycorrhizal fungi (38%) were the bush land sites (Site E and Site F). The lowest level of colonisation of roots by mycorrhizal fungi was at Site C (7%). Phosphorus was highest in soil from Site C (61 P $\mu\text{g/g}$ soil) and lowest in soil from Site F (19 P $\mu\text{g/g}$ soil).

The next step is to re-sample the same sites on a regular basis to determine whether there are any consistent patterns in the relative abundance of these organisms. This can be tracked with information about how the sites are managed to determine trends and any significant changes over time. With ongoing monitoring of these soils, the types of mites, springtails and mycorrhizal fungi present will become familiar. Questions about their relationships with each other and environmental factors will also become clearer, such as with seasonal patterns in weather, changes in soil type, phase in the farming cycle, and with management practices. Further monitoring will be necessary to clarify these responses.



Figure 10 The phase in the farming cycle will influence the relative abundance of mites and springtails as well as the abundance of mycorrhizal fungi inside roots. (Photos: L Abbott)

7.0 References and Websites

General information on soil health and natural resource management - Wheatbelt Natural Resource Management

<http://www.wheatbeltnrm.org.au>

Extracting soil fauna

<http://www.qm.qld.gov.au/Find+out+about/Animals+of+Queensland/Insects/Collecting+insects/Tullgren+Funnels>

SPICE website – Soil Science Resource

<http://spice.wa.edu.au/spice-initiatives/soil-science/>

Monitoring Soil Science Project (for schools)

<http://soils.duit.uwa.edu.au/>

Spice – Soil Life Resource

<http://spice.wa.edu.au/soil-life/>

Podcast – Soil Sampling

<http://soils.duit.uwa.edu.au/mediafiles/soilsampling/index.php>

Podcast – Extracting Soil Fauna

<http://soils.duit.uwa.edu.au/mediafiles/extractingsoilfauna/index.php>

Staining Mycorrhizal Fungi

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* 64: 5004–5007

Brundrett MC. 2008. Mycorrhizal Associations: The Web Resource.

<http://mycorrhizas.info/vam.html>

Introduction to soil health

<http://soilhealth.com>

Information for soil health

<http://soilhealthknowledge.com.au/>

Information on soil quality and health across Australia

<http://www.soilquality.org.au>

8.0 Contacts

Wheatbelt Natural Resource Management

Phone: (08) 9670 3100

Fax: (08) 9670 3140

Email: info@wheatbeltnrm.org.au

Georgie Troup

Project Manager Sustainable Agriculture

Wheatbelt Natural Resource Management

Email: gtroup@wheatbeltnrm.org.au

Dr Guy Boggs

Program Manager Sustainable Agriculture

Wheatbelt Natural Resource Management

Email: gboggs@wheatbeltnrm.org.au

Professor Lyn Abbott

School of Earth and Environment, Faculty of Science
and UWA Institute of Agriculture

The University of Western Australia

Email Lynette.Abbott@uwa.edu.au

Dr Zakaria Solaiman

School of Earth and Environment, Faculty of Science
and UWA Institute of Agriculture

The University of Western Australia

Email Zakaria.Solaiman@uwa.edu.au







wheatbelt natural resource management

PO Box 311
Northam WA 6401

Tel: 9670 3100

Fax: 9670 3140

Email: info@wheatbeltnrm.org.au

Web: www.wheatbeltnrm.org.au

Facebook.com/wheatbeltnrm

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