Australia's National
Science Agency

DRAFT Monitoring Protocols Manual for the MER Pilot Network

Soil Sampling Module

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Contributions

J. Hodgson led the coordination, drafting and editing of this document. She also led the field trials and contributed to the design of the protocols. S. Prober, B. Sparrow, L. Broadhurst led the design of the protocols and provided revisions. L. Broadhurst also assisted in conducting field trials. N. Gellie and the TERN protocols team provided drafts, revisions and conducted field trials of the protocols. J. Carwardine and S. Nicol provided revisions.

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CSIRO and TERN acknowledge the traditional country and custodians of the lands on which we operate. We pay our respects to their ancestors and their descendants who continue the connection to Country. We celebrate the stories, culture and traditions of Aboriginal and Torres Strait Islander Elders of all communities who also work and live on this land.

Contents

Introduction

Soil chemistry and biology were identified as highly important factors of soil by ecological experts and practitioners at the MER Network consultation workshops. They were also recognised as an often under studied aspect of ecological monitoring. It is planned that soil sampling will occur at the first monitoring event for a site and then be stored as a baseline resource for others i.e. academic researchers who become involved in the network to utilize and expand upon. Therefore, soil sampling by Service Providers will only occur in the first year of monitoring.

It is important to be aware that the biggest driver of physical and chemical soil patterns is the occurrence and cover of trees and shrubs or other distinctive microhabitats. Therefore, it is important to sample these variations accordingly. It is also important to consider recent weather events at the time of sampling. For example, wet samples are difficult to process and curate so recent significant rainfall may cause you to reconsider the timing of your sampling.

This Soil sampling module details two field protocols and one protocol that details the preparation and curation of samples outside the field. The two field protocols can be carried out concurrently. To save time in the field it is recommended that you pre-label and fill collection bags with silica as detailed in part three.

Part One describes the protocol of soil sampling for soil chemistry properties. The soil chemistry survey will collect samples that are standardized and suitable to be analysed for key nutrients and physical characteristics.

Part Two describes the protocol for biological soil sampling. The Bbiological soil sampling survey follows a similar sampling protocol to soil chemistry with slight variation that enables investigation of the soil microbial composition of the plot.

Part three describes the preparation, storage and processing of the soil samples collected in part one and two.

It is assumed that the plot set up has been completed and tapes have been laid out prior to completing these modules.

Glossary

Term	Definition
Site	A Site is a cluster of one set of plots. These may occur in a single reserve or property or be spread across areas with differing tenures.
Plots	Plots are within sites and each plot falls into one of the two or three experimental treatments.
Blocks (Triplets or Pairs)	Within a Site, all plots are grouped into either a Triplet or Pair (depending on whether two or three treatment types exist for the Site). One plot in each Triplet (or Pair) represents one of the treatment groups. Also referred to as a 'Block.'
Transect	A transect is a defined linear collection area within a plot. All MER Network plots have the same transect setup defined by a tape measure and have length (e.g. 10m, 25m) direction (e.g. north, south, east and west) and sequence (e.g. South 1, South 2, South 3 etc.). This information (set-up) is important for all MER Network protocols.
Protocol	The standard procedure used to monitor a MER Network Plot in rangeland and open forest.
Soil sample	A soil aliquot taken from bulked soil sub-samples at sub-sample sites (e.g. n=36 for 25m plot and n=25 for a 10m plot). The soil sample weight is determined by its allocation (e.g. 500gm for soil chemistry or 200gm for soil biology).
Soil sub-sample site	The soil sub-sample site is defined by intersections made on tape transects during plot setup. Although sub-sample sites can be in the vicinity of the tape intersection as it is easier to count them (e.g. n=36 for 25m plot and n=25 for a 10m plot) more importantly the sub-sample sites should be used to include as much microhabitat variation as is possible at the plot (e.g. under trees, open areas, below grasses and shrubs etc.)
Soil sub-sample	A soil sub-sample is a single point sample from the plot. The soil chemistry sub-sample is representative of the soil down to 10cm at the sub-sample site. The soil-biological sub-sample is representative of the top 3cm of soil at the sub-sample site.

Part I Soil chemistry samples

Analysis of soil chemistry can reveal the levels of key nutrients in bio-available forms present in the soil at a designated point in time. The soil type and its nutrient levels can often be a key determinant of vegetation distribution and composition. Fire often leads to significant changes in soil chemistry as a result of ash, carbon burn-out or issues such as soil erosion brought on by fire and climate, therefore it is an important variable to consider when monitoring vegetation after fire.

The aim of the sampling is to capture the natural variability of the plot and then bulk and mix to obtain a representative soil sample that can then be stored and analysed at a later date by interested parties of the Network. Because soils are stored, it is important that soils are processed (dried) quickly to limit the amount of chemical change.

1 Soil sampling for soil chemistry properties

1.1 Equipment list

- Bucket auger or other soil sampling tool to be advised (e.g. trowel)
- Silica beads; below is the minimum needed per plot
    - white silica beads 90% blue indicators 10% = 125gm per bag per plot
    - spare to allow for regular changes
    - Note: do not dispose of spent silica beads as they can be dried out and reused
    - Refer to manufacturers material Safety Data Sheet in section 3 for handling and storing silica beads
- Medium (ca 30 x 20cm) white block durable zip-lock bags x number of plots (it is recommended to pre fill these with silica before sampling see section 3.1 for preparation details)
- Medium calico bags x number of plots
- Large calico/chaff bags (ca 50 x 30cm) x 1 per site to hold all chemical and biological samples
- Persola/scales or pre-prepared 500 g and 200g soil weighted bag as reference)
- Permanent marker/ labels
- Personal Protective Equipment PPE (e.g. hat, sunscreen, protective clothing and gloves)

1.2 Procedure

1. Label all plastic and calico sample bags and place silica in the plastic bags (then seal) as described in Section 3.1.

> TIP: We strongly recommend soil bags are prepared before going into the field, including labelling and silica gel. Remember to seal the bags once silica gel is added to avoid unnecessary dust and avoid exposure of silica to moisture.

2. Beginning in the southwest corner of your plot you will use the soil sampling tool to take 36 sub-samples to a depth of 10cm in a grid across the plot (See Figure 1.1). The grid pattern automatically accounts for varying microhabitats and prevents biased sampling. Use the grid to define sub-sample numbers (e.g. n=36 for 25 m plot and n=25 for a 10 m plot) and sub- sample as many microhabitats as possible in that plot.
3. When you arrive at a sub-sample site remove loose leaf litter, scats and vegetation from the soil surface then excavate and remove the subsample.

TIP: If using a trowel, ensure your sub- sample is cylindrical or as 'vertical-sided' as possible so that there are equal amounts of soil from the top (0 cm) through to bottom (10 cm) of the sample. This may require digging a wider hole and sub-sampling across its face to 10cm. Note one tablespoon (e.g. 25 g) from each sub-sample is sufficient for the bulked sample.

4. Aim for clean sub-samples free of all rocks and debris.
5. Empty the sub-sample (e.g. bucket auger contents or trowel contents) with the soil into a labelled calico sample bag and mix. Once all sub-samples have been collected mix the soil and remove excess to ensure there is at least 500 g of soil for the sample by using scales or pre-prepared soil weight reference bag.
6. Roll the calico bag up and secure with attached ties then place this calico bag in your prepared and labelled larger snap lock bag with half a cup of mixed silica granules (see section 3.1 for preparation details). Close the bag push the air out and snap it tightly shut.
7. Place all labelled plot sample bags in a labelled medium calico/chaff site bag (including date, site and plot IDs) then keep the bag cool (in an Esky or fridge) until the samples can be processed (see section 3.3 for details on processing).
8. Silica will need to be checked regularly and changed until the self-indicating granules retain their original colour. A change in colour from blue to pink of the self-indicating granules reveals its moisture absorbing capacity has been reached and it needs to be replaced with fresh silica (see Section 3.2 for storage and curation details). Once samples are dry they should be kept cool but don't need to be refrigerated.

TIP: If your soils are quite wet and you have access to a drying oven (not one used for food), drying your soils in the calico bags (remove from the plastic bag with silica) for several days at 60°C will save the additional time needed for changing silica. Research organisations, universities or CSIRO in your area may be able to assist.

9. Once a plot is collected check off the soil chemistry protocol found in the Fulcrum app.

1.3 Plot layout

For a plot of 25 m x 25 m, a total of 36 samples should be collected in a grid across an area of 25 m x 25 m in the south-west corner of the plot as shown in Figure 1.1a. In a 10 m x 10 m plot, 25 samples should be collected in a grid across the whole plot as shown in Figure 1.1b.

The aim of using the grid is to ensure different microhabitats (e.g. gaps, beneath trees, beneath shrubs, under litter or bare soil) are sampled in proportion to their occurrence on the plot. Thus, avoid moving away from thick vegetation, shrubs etc. if they are at your point location.

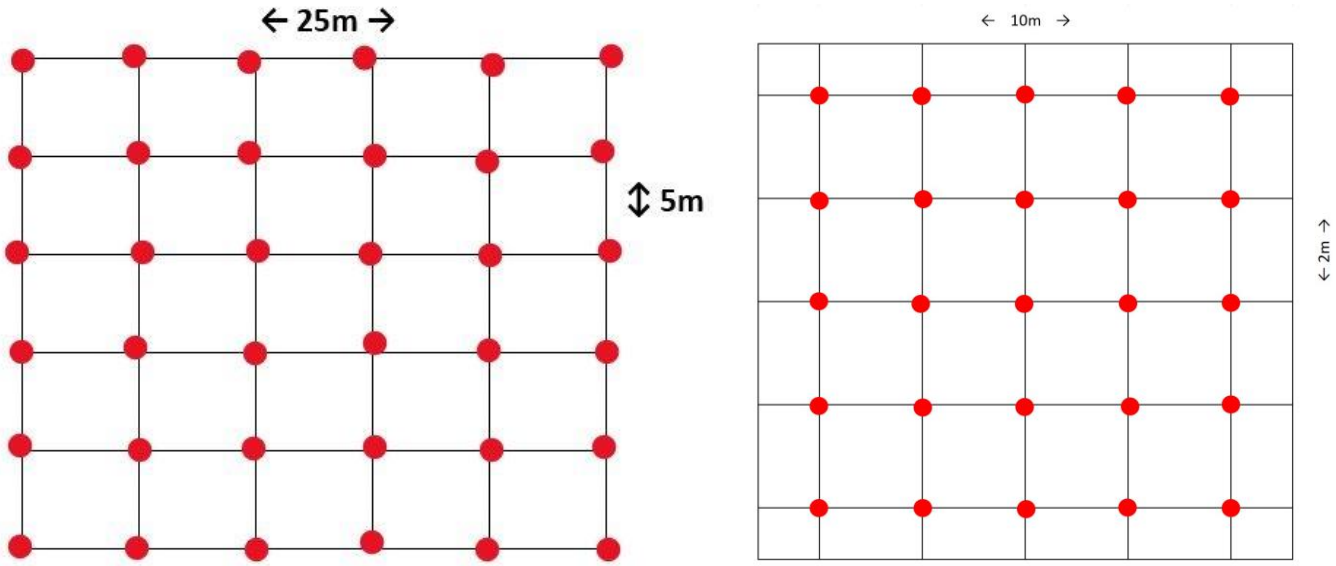

Figure 1.1 a) Soil sampling distribution for plots of 25 m x 25 m. b) Soil sampling distribution for 10 m x10 m plots. NB: taking samples from the exact locations above is not important, because the grid distribution is intended to provide a framework to collect the desired number of sub-samples that is representative of the distribution of microhabitats across the plot.

Part II Soil biological samples

Analysis of soil biological properties, i.e. the genetic material present in the soil, can help us to understand which microbial organisms (e.g. bacteria, archaea and fungi) are present in the plots. Significant changes in microbial composition over time or between treatments (plot types) can help predict the trajectory of the vegetation community above ground.

The aim of the sampling is to capture the natural variability of the plot with a mixed bulk sample collected from across the plot. This sample can then be stored and analysed at a later date by interested parties of the Network. Because soils are stored, it is important that soils are processed correctly to preserve the genetic material in the sample.

2 Soil sampling for soil biological properties

2.1 Equipment List

- Silica beads as per previous section; below is the minimum needed per plot
    - white silica beads 90% blue indicators 10% = 125 g per plot
    - spare to allow for regular changes
    - Note: do not dispose of spent silica beads, they can be dried out and reused.
    - Refer to manufacturers material Safety Data Sheet in section 3 for handling and storing silica beads
- Nitrile gloves
- Medium (ca 30 x 20cm) white block durable zip-lock bags x number of plots (it is recommended to pre fill these with silica before sampling see Section 3.1 for preparation details)
- Medium calico bags x number of plots
- Permanent marker/labels
- Metric trowel or paint scraper with 3 cm pre-marked
- Persola/scales to check soil weight or pre-prepared 200 g soil weight reference
- Sanitising equipment for trowel
    - alcohol wipes and/or spray
    - scrubbing brush

2.2 Procedure

Using the same grid distribution as shown in Figure 1.1;

1. Prepare bags and silica as per Section 3.1, before you go in the field.
2. Offset sub-sampling area from previous soil chemistry sub-sample sites by at least 30cm
3. Clean and sanitize the trowel between use at different plots by
    i. remove debris using a brush
    ii. spray and/or wipe with ethanol spray/alcohol wipes
4. Gently remove any loose material (i.e. leaf litter, scats, biocrust) from the soil surface with trowel.
5. Use a sanitized trowel or paint scraper collect a small sample of soil surface to a depth of 3 cm. Ensure your sub- sample is cylindrical or as ‘vertical-sided’ as possible so that there are equal amounts of soil from the top (0 cm) through to bottom (3 cm) of the sample.

When bulked the whole sample should be at least and approximately 200g. (e.g. 15 g or tablespoon of soil crust from each sub-site). We recommend pre-preparing a soil bag of this weight as a field guide.

4. Aim for clean sub-samples free of all rocks and debris.
5. Empty the sub-sample into a labelled calico sample bag and mix. Once all sub-samples have been collected mix the soil and remove excess to ensure there is at least 200g of soil for the sample by using scales or pre-prepared soil weight reference bag.
6. Roll the calico bag up and secure with attached ties then place this calico bag in your prepared and labelled larger snap lock bag with half a cup of mixed silica granules (see section 3.1 for preparation details). Close the bag push the air out and snap it tightly shut.
7. Silica will need to be checked regularly and changed until the self-indicating granules retain their original colour. A change in colour from blue to pink of the self-indicating granules reveals its moisture absorbing capacity has been reached and it needs to be replaced with fresh silica (see Section 3.2 for storage and curation details). Once samples are dry they should be kept cool but don't need to be refrigerated.
8. Place all labelled plot sample bags in a labelled medium calico/chaff site bag (including date, site and plot IDs) then keep the bag cool (in an Esky or fridge) until the samples can be processed (see section 3.3 for details on processing).

TIP: If your soils are quite wet and you have access to a drying oven (not one used for food), drying your soils in the calico bags (remove from the plastic bag with silica) for several days at 60°C will save the additional time needed for changing silica. Research organisations, universities or CSIRO in your area may be able to assist.

9. Once a plot is collected check off the soil chemistry protocol found in the Fulcrum app.

Part III Soil sample processing

Explanation of soil bag preparation, drying procedure and where to submit samples.

Image credit: TERN

3 Soil processing protocol

3.1 Preparing your soil sample bags prior to sampling

Silica gel can absorb 40% of its weight in moisture so it is an excellent desiccant but may cause irritation, and should be handled with care and in a well ventilated area. This includes wearing appropriate protective eyeglasses, gloves, and protective clothing. Also wear an approved respirator if handling significant amounts or if irritation is experienced. Store away from food or beverages in tightly closed containers in a cool, dry and well-ventilated area.

Read material safety data sheet here and procedure below before handling silica beads.

Procedure (preferably do this before going into the field)

1. Silica will take on atmospheric moisture so containers should not be left open for any longer than necessary. Decanting is best done as close to sampling as possible especially in humid or damp environments.
2. Prepare a safe well ventilated working area with enough space to undertake silica decanting
3. Label (plot ID, site name, date, collector, sample type (chemical or biological)) enough medium zip lock and medium calico bags for you site (e.g. 16 for 8 plots and 24 for 12 plots).
4. Label one suitable sized large calico/chaff site bag to carry all the full plot bags above. (e.g. ca 10 kg for 8 plots and ca 15 kg for 12 plots of sample soil and silica)
5. Put on the PPE recommended in the silica bead manufacturer Safety Data Sheet (e.g. eye, skin and respiration protection as per Australian Standards AS/NZS 1715 and 1716)
6. Place a suitably sized airtight receptacle (20L bucket) on your working area. (e.g. silica 4 kg for 8 plots, 6 kg for 12 plots as a minimum, or as needed)
7. Decant silica from manufacturer's tubs with a dedicated measuring beaker at a ratio white silica 90%, blue indicator silica 10% and mix gently (e.g. 10 kg mixed = 9 kg white and 1 kg blue) then seal all containers
8. Decant 125 g of the mixed silica into air tight labelled zip lock bags with aligned labelled calico inside and seal thoroughly.
9. Place all the prepared plot bags in a labelled site bag ready for soil collection

3.2 Drying and storing your soil samples

Some environments have high atmospheric moisture whilst others may have had recent precipitation. This moisture will degrade samples so it is important to curate the samples well

prior to them being dispatched to the central project soil archive in Adelaide. The indicator beads will indicate when the samples are sufficiently dry to send.

Procedure

1. Usually after 2-3 days, indicators will begin to change colour. If they change colour, prepare a safe well ventilated working area for replacing the beads.
2. Using the same PPE procedure as stated in section 3.1, empty spent silica in to a suitably sized empty airtight receptacle (20 L bucket).
3. Replace with 125 g of prepared mixed silica from 3.1 into bags and seal bags thoroughly.
4. The number of times you will need to replace silica will depend on how moist the soil is. You can use more silica if the soil is very wet, or if the soil is dry you may not need to change it at all. The soil is sufficiently dry when the silica no longer changes colour, and no longer need to be refrigerated. Once dry, keep soils in a cool place until you are able to post them (preferably as soon as possible).
5. See below for quarantine requirements and details for posting soils to Adelaide.
6. Silica can be reactivated by drying in an oven at ca 110 Celsius for a couple of hours or until the indicator colour reappears. However, ovens used for food preparation are not suitable for this process and the manufacturers PPE recommendations should be followed at all times when handling silica.

3.3 Sending your soil samples to the soil storage facility

Both the soil chemical and biological samples must meet the following criteria before sending for storage at the TERN facility at the University of Adelaide Waite Campus.

1. All samples must have the following information accompanying them

- Plot name
- Site name
- Name of contact person associated with sample collection
- Date of soil collection
- Whether it is the chemical or biological sample
- Mark all bags before sampling, including on the outer plastic and inner calico bags

2. Quarantine forms and permissions need to be completed in a two stage process:

a. Obtain Plant Health Import Certificate from your MER contact

- First, fill out your local information on the Plant Health Import Certificate application provided (use our example as a guide). Note the form is formally submitted by the TERN in Adelaide, so we have included their details as the applicant. You will need to add information on the source locations, number/weight of each type of sample being sent. You will also need to estimate within 3-4 days range of when the soils will arrive at their destination (which can be amended later if necessary).

- Send the application by email to Nick Gellie (Nick.Gellie@adelaide.edu.au) and wait until TERN get back to you with the certificate (usually within a day or two).
- Print the certificate and attach to the final soil package as described below.
- Note that each application costs $110, so we suggest waiting until you are ready to send all of your soil samples for a sample season at once.

b. Fill out and sign the 'Soil Source Declaration' and email it to Nick Gellie (Nick.Gellie@adelaide.edu.au).

3. Samples need to be triple bagged for quarantine purposes. We recommend the following:

- Leave (or place back) each calico bag of dry soil back into its original labelled plastic bag (with silica removed) and seal (taking care to remove air for easier packing). These are then all double-bagged.
- Place the 8 soil chemistry samples and the 8 soil biological samples from a site into two separate larger calico bags and label them. This makes it easier for the receiver to processes samples. If you have more than one site, do the same for your other sites.
- Place all samples you are intending to post into a larger bag, e.g. your chaff bag.
- Attach the "Plant Health Certificate" to the outer bag.
- Attach the filled and signed "Soil Source Declaration" form and attach to the outside of the bag.
- Please take a photograph of the final bag with the label attached as sometimes PIRSA request evidence it was attached.

Owing to some changes currently occurring in South Australian quarantine, please contact Nick Gellie to check for updates prior to sending your samples.

When you are ready to send samples to the University of Adelaide, please post to:

Attn: Dr Nick Gellie (MER Network sample),

The University of Adelaide Waite Campus,

Goods Receiving, Wine Innovation Central Building, corner of Paratoo Road and Hartley Grove Urrbrae, SA 5064,

Australia.

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