

Soil nitrification

DON'T FORGET

EQUIPMENT: Spade; 2 mm sieve; 500 ml plastic food containers and lids x 50; aluminium foil; plastic bags for soil sample; plastic funnels; filter papers; portable balance; distilled water; squeeze bottle; thermometer (0–50 or 100 °C); millivolt meter; nitrate and reference electrodes; standards; buffer; polythene sheet; pencil and paper; oil/water-sensitive papers; permanent marker pen.

A composite soil sample is required that is representative of soil in the treatment area. Ensure the soil type and cover vegetation is the same before digging sub-samples at bullet point 1. If there are distinctly different soils present in the treatment area, either choose the dominant type (recommended) or decide to test more than one soil.

Method

- Dig 1 kg surface soil (0–5 cm deep) from 10 sites in the area to be sprayed. Remove vegetation/roots before passing through a 2 mm soil sieve. If wet, air-dry soil a little until it can be sieved. Mix the samples together to produce a composite sample.
- Weigh 100 g soil aliquots into 40 pre-weighed plastic food containers and fasten lids. Double bag 1 kg of composite soil to assess later the texture, pH and water-holding capacity.
- Determine the soil moisture and water-holding capacity of the soil using the method sheets from chapter 5. Calculate 70% of water-holding capacity (water-holding capacity in g x 0.7).
- Wet the soils in the food containers with a solution of ammonium sulphate¹ to bring the nitrogen amendment to 100 µg NH₄-N g dry weight of soil and the soil moisture to 70% of field capacity. Now make a note of the soil weight plus container and write it on the container (not lid) in permanent marker pen.
- A short time before spraying, place 40 containers at sites within the area to be sprayed. This might be between crop rows or in the open if spraying is aerial. Arranging them in clusters of 4 is convenient for handling and logistics – perhaps 1 m apart. Also place magnesium oxide slides or water/oil-sensitive papers around each cluster of containers.
- Just before spraying remove the lids of half of them (20). Those with their lids left on (20) are designated the controls and are marked as such. In the absence of shade, place aluminium foil on the lids of the controls to reduce the heating effect.
- Replace lids as soon as possible after spraying – magnesium oxide slides and papers should show droplet deposition and confirm soils contaminated with pesticide (see chapter 4 for method to count droplets) – to reduce evaporation of soil water and bring samples to the field camp or laboratory.
- Place all the containers with their lids removed in a stout cardboard box lined with a polythene sheet. Place a few containers of water in the box to increase the relative humidity and locate the box(s) in the shade.
- Adjust soil moisture gravimetrically in all containers *daily* by placing containers on a balance and dropping water (evenly) on to the soil until the weight at fourth bullet point is reached.
- Incubate soils for up to 50 days (depending on ambient temperature) and destructively sample four treated and four controls at, e.g. days 0, 10, 20, 30 and 40. The cooler the temperature the longer the sampling period.
- Before extracting NO₃-N from soils, weigh the container and soil and then mix soil with a spatula or spoon. Take 50 g sub-samples from each of the replicates, place in a bottle and shake for 30 s with 100 ml of deionized/distilled water. Allow to settle for 30 min before repeating shaking and settling. Shake and settle a third time, then take 10 ml of supernatant and mix it with 10 ml of 2M (NH₄)₂SO₄ – an ionic strength adjustment buffer or ISAB². This is the sample that will be measured by the ion-selective electrode. Make sure your samples are labelled at all stages.

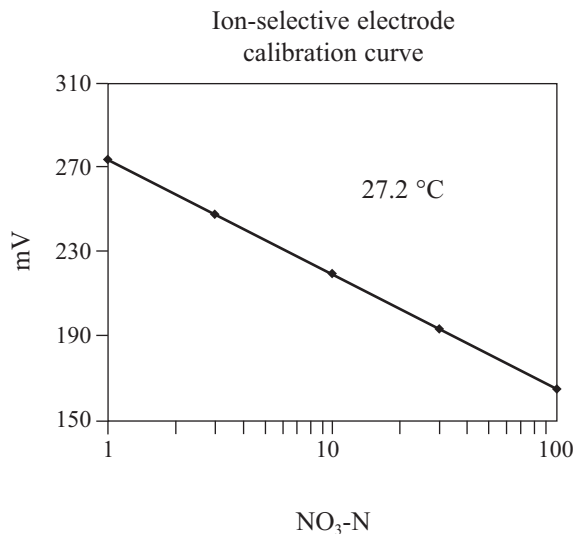
- Prepare a series of $\text{NO}_3\text{-N}$ standards³ (from 1 to 100 ppm) to calibrate the ion-selective NO_3 electrode coupled with a double junction reference electrode. Take 10 ml of each standard and add 10 ml ISAB prior to calibration. Standards and samples must be at the same temperature for measurement of millivolts (store samples and standards in a tray of water if in the field). Read off the millivolts and plot mV against concentration of the standard and then calculate the nitrate in samples as $\mu\text{g NO}_3\text{-N/g}$ dry weight of soil. Wash electrode between samples with distilled water. It does not matter if the measurement differs at the next sampling interval, which may be 10 days later, provided all samples and standards are at the same temperature when they are measured.
- Recalibrate the electrode at every sampling period, discarding the standards unless they were refrigerated.
- Average the controls and treatments for each time interval and graph the results of control and treatment as cumulative $\text{NO}_3\text{-N/ g}$ dry weight of soil against time in days (x axis).

Notes

¹ Ammonium sulphate for nitrogen amendment: dissolve 4.716 g of dry $(\text{NH}_4)_2\text{SO}_4$ in 1 litre of water. If 10 ml of water is required to bring the 100 g soil sample to 70% field capacity in step 3, then using 10 ml of the $(\text{NH}_4)_2\text{SO}_4$ solution to wet the soil will provide 47.16 mg $(\text{NH}_4)_2\text{SO}_4$, i.e. 10 mg $\text{NH}_4\text{-N}$ or 100 $\mu\text{g NH}_4\text{-N/g}$ soil.

² ISAB: 2M $(\text{NH}_4)_2\text{SO}_4$. Dissolve 264 g of $(\text{NH}_4)_2\text{SO}_4$ in 1 litre of water.

³ $\text{NO}_3\text{-N}$ standard: 100 $\mu\text{g NO}_3\text{-N ml}^{-1}$. Dry pure KNO_3 , weigh out 0.722 g and dissolve in deionized water and dilute to 1 litre in volumetric flask. Keep cold (may last 1 month) otherwise make up fresh. Make 1, 5, 10 and 50 $\mu\text{g NO}_3\text{-N}$ standards by pipetting 0.5, 2.5, 5 and 25 ml of this standard into labelled volumetric flasks (50 ml) and making up to volume. Take 10 ml of each standard, add 10 ml ISAB and measure the potential in mV. Plot on a semi-log paper to obtain a more or less straight line.



Soil respiration (long-term *in situ*)

DON'T FORGET

EQUIPMENT: Plastic bags for soil sample; distilled water; squeeze bottle; thermometer; catering size coffee cans (approximately 25 x 28 cm); screw-cap glass jars (6 x 7 cm) with air-tight lids; wire tripod; hatchet; aluminium foil; cardboard boxes; notebook; permanent marker pen; 1N NaOH.

Barium chloride is poisonous – do not pipette by mouth.

Try to match the sites as closely as possible in terms of soil type, surrounding vegetation and cover (see text in chapters 1 and 7 matching sampling sites).

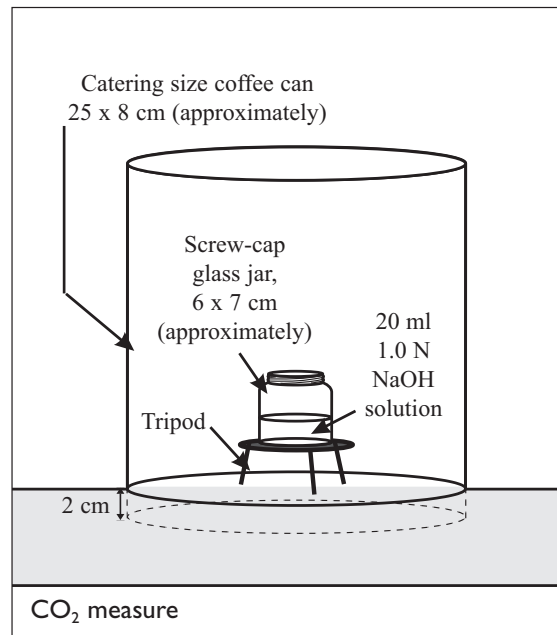
Method

- Choose 5–10 matched sites for the long-term measurement of soil respiration in each of the treated and untreated areas.¹
- When at each site (not in advance) carefully pipette 20 ml (use a pipette bulb) of 1N NaOH into a glass jar placed on a wire tripod that holds the jar off the ground by about 2 cm (see inset). Place the coffee can over the jar and grind it into the soil to a depth of about 2 cm. If the site is exposed to direct sun, provide shade with a twig and grass roof or aluminium foil folded around cardboard and placed on top of the can. Note the time and allocate a site number in a notebook.
- At the same time, set two jars of NaOH inside identical but sealed coffee cans (the plastic lids are normally sufficient but a really good seal can be achieved by smearing silicone grease around the edge of the lids). Expose them under the same conditions as they will act as controls for the measurement of carbon dioxide in air trapped initially within cans.
- Return after 24 h, carefully lift the cans from the soil and collect the jar of NaOH. Cap with an air-tight lid, number the jar (not the lid) with a permanent marker pen and note the exposure time. Store the jar carefully for transport to the camp or laboratory.
- Re-set the monitoring apparatus a short distance away from the previous sites to continue monitoring over a fixed period, e.g. day 1, 2, 3, 5, 10, 20 and 30 after spraying.
- Using a Pasteur pipette, add enough 3N BaCl₂ to the NaOH solutions in the jars to precipitate BaCO₃ (a white precipitate) and then add a few drops of phenolphthalein (coloured indicator). Titrate the NaOH *slowly* with 1N HCl while swirling the jar gently taking care to avoid contact of the acid drops with the precipitate (the BaCO₃ precipitate must not dissolve). Note the titration point where the indicator changes colour.
- Calculate the carbon dioxide released from the soil using the formula:

$$\text{mg CO}_2 = \frac{(\text{ml HCl titrated for sealed cans} - \text{ml HCl titrated for cans exposed to soil})}{\text{I (normality of acid)} \times 22 \text{ (equivalent weight of CO}_2)}$$

$$\text{then convert to mg CO}_2 \text{ m}^{-2} \text{ h}^{-1} = \frac{\text{mg CO}_2 \times 10,000 \text{ cm}^2}{\text{area of soil enclosed (cm}^2) \times \text{hours of exposure}}$$

- Calculate the mean respiration rate (+/-SE) for replicate samples and graph the results of carbon dioxide released against time (x axis).



OTHER CONSIDERATIONS

¹When choosing sites, ensure that you enclose similar areas of ground avoiding roots of trees, grass and other vegetation (in crop areas place between plants), ant and termite holes. Never repeat in a short space of time at the same place.

Reagents:

N NaOH Dissolve 40 g of NaOH in 500 ml H₂O and dilute to 1 litre

N HCl Dilute 83 ml of concentrated HCl (37%) in 1 litre of H₂O

3N BaCl₂ (poisonous) Dissolve 31 g of anhydrous BaCl₂ in 100 ml of H₂O

Phenolphthalein: 1 g in 100 ml 95% ethanol.

Carry all reagents in secure containers – plastic is best for these – check lids!

Soil respiration (semi-continuous)

DON'T FORGET

EQUIPMENT: Spade; 2 mm sieve; 500 ml plastic food containers and lids x 50; aluminium foil; plastic bags for soil sample; portable balance (200 g); distilled water; squeeze bottle; thermometer; 0.5 mm sieve or pre-weighed straw material; Draeger tubes (0.02–0.3% CO₂) and bellows; modified lid for measurements; permanent marker pen; stop-watch or wrist-watch with minute hand.

A composite soil sample is required which is representative of soil in the treatment area. Match the soil type and surrounding vegetation before digging sub-samples at first bullet point. If there are distinctly different soils present in the treatment area, either choose the dominant type or decide to test more than one soil.

Method

- Dig 1 kg surface soil (0–5 cm) from 10 sites in the area at risk from pesticide, mix them together and remove vegetation/roots before passing through a 2 mm soil sieve. If too wet, air-dry until it can be sieved.
- Weigh 100 g aliquots into 40 pre-weighed food containers and apply lids.
- Double bag 1 kg of soil to assess soil texture and measure pH either in the field (see method sheets for chapter 5) or on return to the laboratory.
- Determine the soil moisture and water-holding capacity using the method sheets in chapter 5. Calculate 70% of water-holding capacity (water-holding capacity in g x 0.7).
- Add 0.5 g of dry organic matter amendment (typically dried grass) (see page 154 of chapter 7) milled to pass through a 0.5 mm sieve to each soil aliquot and mix thoroughly.
- Bring the soil moisture to 70% of field capacity by slowly adding distilled water. Now make a note of the soil weight plus container and write it on the container (not lid) using a permanent marker pen.
- A short time before spraying, place 40 containers at sites within the area to be treated. These can be placed in clusters of 4 or spaced more widely over the treated area. Also place water/oil-sensitive papers around each container to estimate pesticide deposition. Just before spraying remove the lids of half of them. Those with lids are designated as the 'controls'. In sunlight, place aluminium foil on the lids of the controls to reduce the heating effect.
- Replace lids soon after spraying to reduce evaporation noting which were exposed (write 'exposed' on the containers) and bring samples to the field camp or laboratory.
- Collect the oil/water-sensitive papers but do not touch their surfaces (see chapter 4 for method used to count droplets).
- Place all the containers with their lids loosened (to allow exchange of air) or removed in a stout cardboard box lined with a polythene sheet to reduce evaporation (incubator). Place a few containers of water in the box to increase the relative humidity and locate the box(es) in the shade.
- Adjust soil moisture gravimetrically in all containers every other day by placing containers on a balance and dropping water (evenly) on to the soil until the weight stated at fifth bullet point is reached. Incubate soils for up to 30 days and measure the carbon dioxide produced (at same time of day) at days 1, 5, 10, 20 and 30 (approximately).
- To measure carbon dioxide, remove a container from the incubator, weigh it to calculate moisture content and then fit a modified lid (see inset) which allows a thermometer access to take soil temperature and air to be sucked over the soil into a Draeger gas analysis tube. Fit the plastic tube to the bellows and pump through 1 litre of air (10 strokes) before snapping off the glass tip (take care) of a 0.02–0.3% carbon dioxide gas tube and placing the plastic tube over the cut end and the other end into the bellows. **Tip:** *Two containers can be linked together with a glass T-piece for this measurement if two special lids are made. This helps to increase the carbon dioxide concentrations when soil respiration rates are naturally low.*
- Draw air into the gas tube by squeezing the bellows *fully* and releasing *fully* 20 times (2 litre), noting the time on a stop-watch at the start and finish, and reading and noting percentage volume of carbon dioxide from the colour development against the scale on the tube. Reset the pump counter, replace the gas tube and start the next container(s).

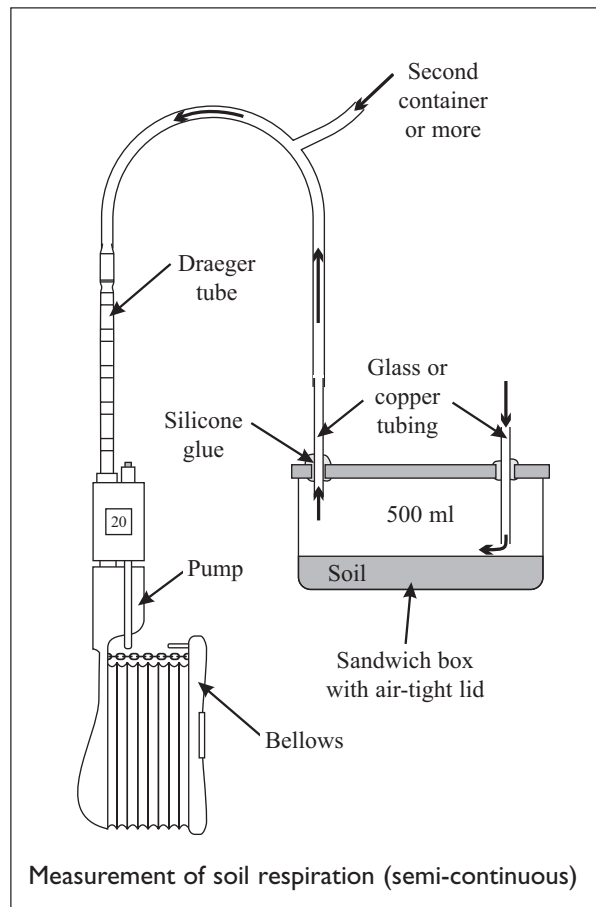
- Every two or three samples, fit a new tube and measure the ambient carbon dioxide concentration and temperature of the air (about 1 m height). When measured at this frequency, air and drawn air temperatures are roughly equal.
- Calculate the respiration rate of soil in each container using the formula:

$$\frac{\% \text{ vol CO}_2 \text{ in sample} - \% \text{ vol CO}_2 \text{ in air} \times \text{vol air drawn through}}{100 \times \text{time taken to draw air (s)} \times \text{g dry weight soil}} \times 3600 = \text{ml CO}_2 \text{ g dry weight soil}^{-1} \text{ h}^{-1}$$

- Correct for STP (standard temperature and pressure):

$$\frac{\text{ml CO}_2 \text{ g dry weight}^{-1} \text{ h}^{-1} \times 101.3 \times 273}{101.3 \times (273 - \text{temperature of soil})}$$

- Once the volume is corrected to STP, multiply by 1.96 to obtain respiration rate in mg CO₂ g dry weight⁻¹ h⁻¹.
- Calculate the mean respiration rate and standard error of four treated and untreated samples (replicates) for each respiration sampling period and plot the results against time (x axis).



Earthworm activity estimation

DON'T FORGET

EQUIPMENT: Trowel or spade; tape measure; string (50 m); pencil and paper; plastic bags; plastic bottles; random number tables or calculator.

Standardize the time of day when counts are made.

TRANSECT METHOD

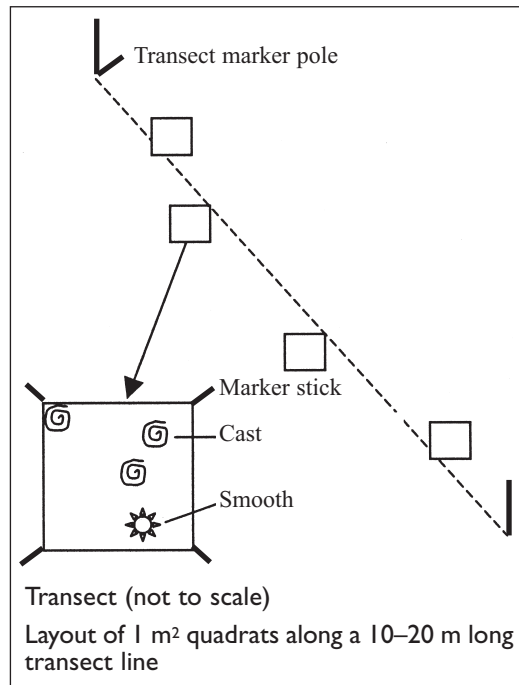
Method

- Identify possible routes for transect lines in treated and untreated areas, selecting ease of access, areas of similar vegetation, slope and general habitat for matching transect routes. Transect lengths will be determined by the size of planted areas in crop land but 10–20 m is a reasonable length in planted fields.
- Use a tape measure or string to lay a straight line, randomizing the starting location of the possible transects.
- Select five to ten m^2 areas to sample for earthworm casts at random intervals along the whole length of the transect line (use random number table or calculator function). When selected, measure and mark the sampling areas to the side of the transect (to prevent walking over them) with string or sticks.
- Count the number of earthworm casts within the sample area and where cast types of different worms are recognizable, count by cast type. Note the numbers on paper against their position along the transect. Also record the weather conditions and time of day.
- Remove or smooth out the casts with the trowel after they are counted.
- Count casts again at anything from 2-day to weekly intervals.
- Take small (200 g) soil samples from plots after observations and keep in sealed, labelled plastic bags for later pH and soil moisture determination.

QUADRAT METHOD

Method

- Where vegetation or crop spacing will allow it, throw 0.25 or 0.5 m^2 quadrats at random up to 10 times (successful landings) in treated and untreated areas. Then follow the same procedures from fourth bullet point of the transect method.



OTHER CONSIDERATIONS

Option: collect and weigh the casts (soil dry weight) to determine the turnover of soil if a long series of observations are planned (e.g. whole season).

Determine the sampling intervals from the relative severity of the pesticide impact on casting.

Express the results as mean number plus standard error of casts per $\text{m}^2 \text{ day}^{-1}$ or other comparable interval. Statistically test for differences between contaminated or treated sites and untreated/uncontaminated sites.

Plot the results of cast rate against time, and percentage soil moisture on the same graph.

Earthworm population estimation

DON'T FORGET

EQUIPMENT: Soil corer; trowel or spade; tape measure; 40% formalin; pencil and paper; rubber gloves; plastic bags; plastic bottles.

Read chapter 3 about how to safely handle formalin.

FORMALIN DRENCH

Method

- Mark out between 5 and 10 sample sites within the treated and untreated areas. Sample size should be about 0.05 m²; size is not critical but standardization of size between sites is. A cylinder of the approximate area can be used to mark the soil at selected sites. In each area to be sampled, dig some soil (200 g) with a trowel, bag in plastic and determine the soil pH and moisture level on site or later in a laboratory.
- Make up the formalin solution carefully (wear rubber gloves and do not splash on skin) by adding 20 ml 40% formalin to 4 litres of water (or 25 ml in 5 litres, 50 in 10 litres, etc.) and mix thoroughly. Measure 300 ml using a graduated cylinder or tin can and pour the diluted formalin evenly over each marked site.
- Collect the worms that emerge. Emergence time varies with soil moisture, earthworm density and proximity to the soil surface: collect at regular intervals (e.g. 15–30 min in moist, organic soils). Place in a plastic bottle labelled with the site name and sample number and add a few millilitres of drench to preserve if processing is not done on site.
- Repeat the drench after worms stop emerging to collect those burrowing deeper in the soil, particularly in drier soils. Sample earthworm populations in the area every 10–14 days, especially when the impact of persistent pesticides like DDT and soil sterilants is being investigated. Do not revisit exactly the same area that was drenched at a previous sampling event.
- Sort the worms into morphospecies and count (seek assistance with taxonomy later). Relate counts to surface area of soil.

SOIL CORES

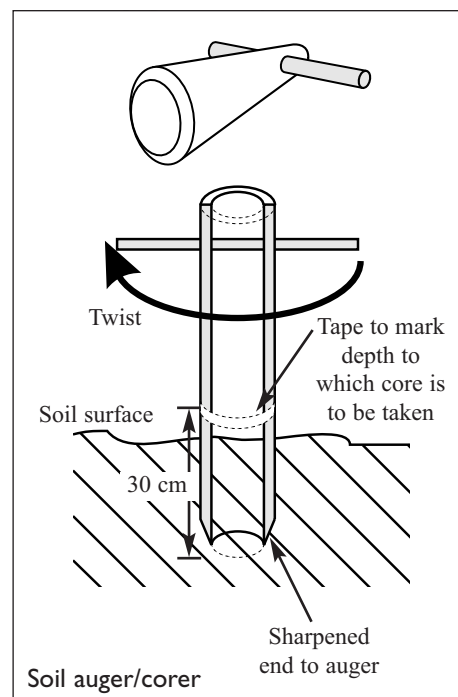
Method

- Take 15–20 soil cores in treated and untreated areas using a steel soil corer to a depth of about 30 cm. Take the cores at random within a sample site.
- Extrude the soil from the corer into a plastic bag if not sorting on site and label (pencil on paper with site name and core number, date, etc., inside the bag).
- Sort the soil on a tray by eye, separating worms into 'morphospecies' groups before counting or weighing. Relate counts to soil volume sampled (density).
- Repeat population estimates every 10–14 days.

SOIL PITS

Method

- Digging soil pits is an alternative to taking cores. Mark out ten 25 x 25 cm plots at random in each treated and untreated area and remove soil with a trowel or spade to a depth of 30 cm (or as required), placing the dug soil in a plastic bag(s) and labelling as described at formalin drench bullet point 3 above.



- Sort the bagged soil by eye on a white tray and separate and count the worms according to type. Express the result as a density (soil volume or surface area).
- Determine the soil pH and moisture content from some of the bagged soil.
- Repeat population estimates every 10–14 days.

OTHER CONSIDERATIONS

A soil corer can be made from 4–6 cm steel water pipe, with the cutting edge sharpened. A larger diameter corer (10 cm+) is preferable.
The formalin drench will kill vegetation so use carefully between crops.
Do not dispose of surplus drench in pools or watercourses.
In drier soils treat larger areas, such as 1 m² samples, with 10 litres drench.
Take soil pH and moisture content of bagged soil soon after sampling (within 1–2 days).

Soil algal cover

DON'T FORGET

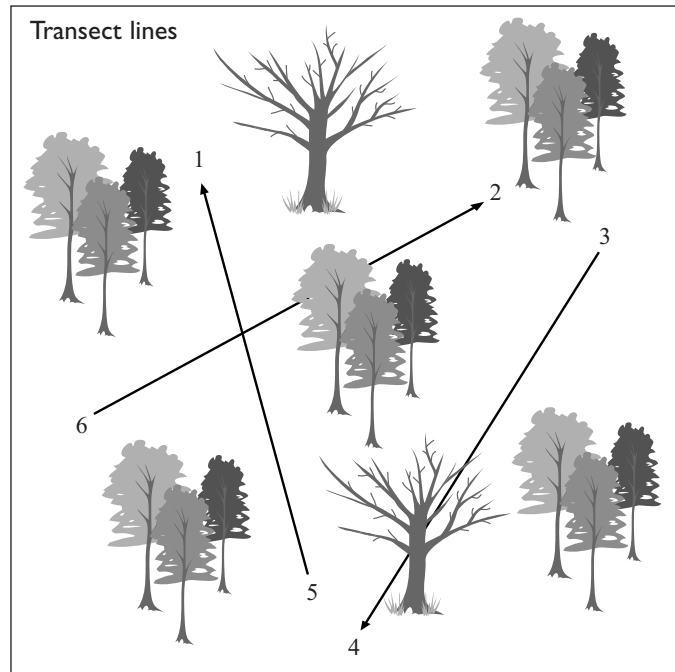
EQUIPMENT: Quadrat; tape measure; string (50 m); pencil and paper; plastic bags.

Transect lines: number the ends of the possible transect lines – there could be many more than the three shown.

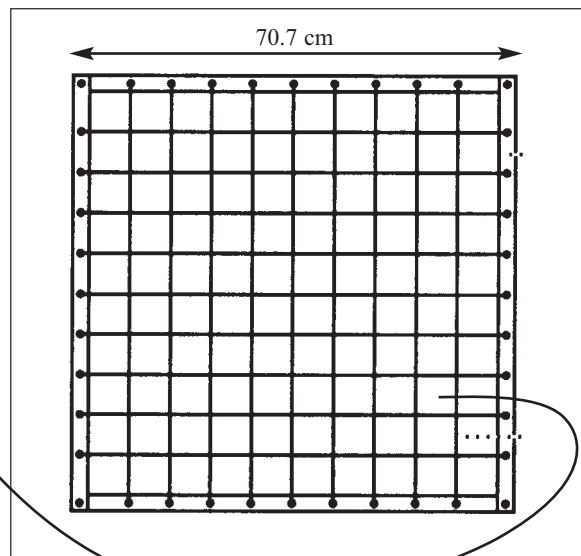
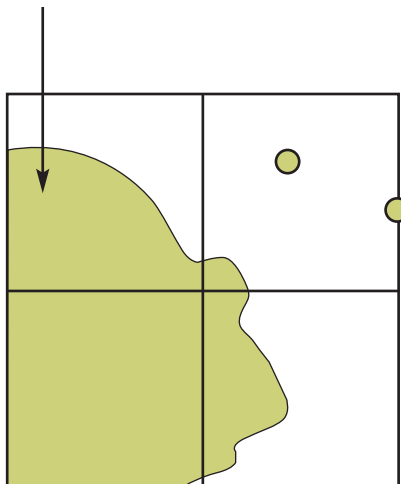
Using random number tables or picking the numbers out of a bag will decide which transect and end to begin. Stopping points along the line can also be generated using tables, assigning distance walked in metres between points.

Method

- Identify possible routes for transect lines in treated and untreated areas, selecting ease of access, areas of similar vegetation, shade and general habitat. About 20–100 m is a reasonable transect length in woodlands and grasslands. Number a few starting points and select the starting location from a table of random numbers. Use a tape measure or string to lay a straight line, using occasional sticks to mark the line (if the string is shorter than the transect line).
- Walk along the transect line stopping at 10 random intervals to place a 1 m or 0.5 m² quadrat to the right side of the transect line (thus forming a belt transect). Ideally the quadrat should be divided into 100 grid squares using tacks and nylon line (see inset).



Mean algal cover approximately 50% in these four squares



Grid square divisions on quadrat

- Assess and note the relative amount of shade from tree canopy, shrubs and open area at the transect points (see method sheets in chapter 5).
- Estimate the percentage cover of algal crusts on the soil using the grid squares: on sandy soils they show up as dark green to black patches. In open savanna grassland, algae may grow just below the surface of sandy soil, showing up as light to dark brown stains.
- Express the results as percentage cover histograms.
- Take samples back to a laboratory in a plastic bag for confirmation of algal presence by examination under a microscope. Wet the sample and leave in the light for a day or two before preparing a slide. Smear the algae very thinly over the surface of the slide, add a cover slip and examine under a high power microscope. Seek the aid of a soil microbiologist or botanist if you cannot recognize algae. It is not necessary to identify them to species.

Litter bags (microbial decomposition)

DON'T FORGET

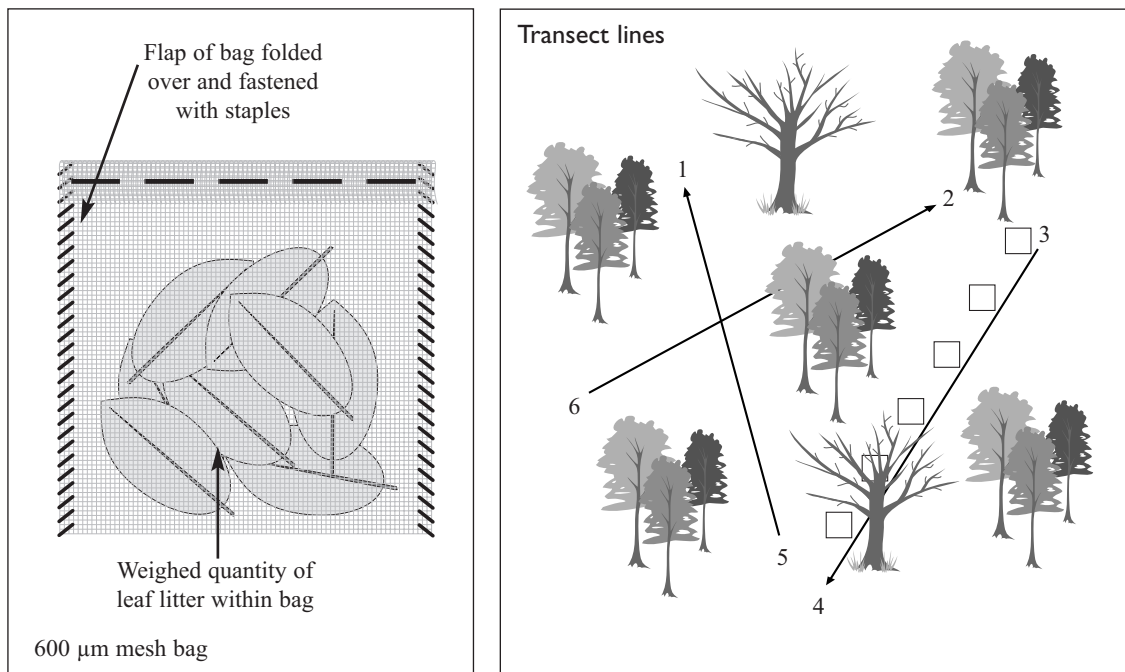
EQUIPMENT: Litter bags; wire; pliers; spade; pick axe; notebook; pencil; labels; thick plastic bags; tape measure; permanent marker pen; paint and paint brush; marker flags or stakes.

IN ADVANCE OF MONITORING EVENT

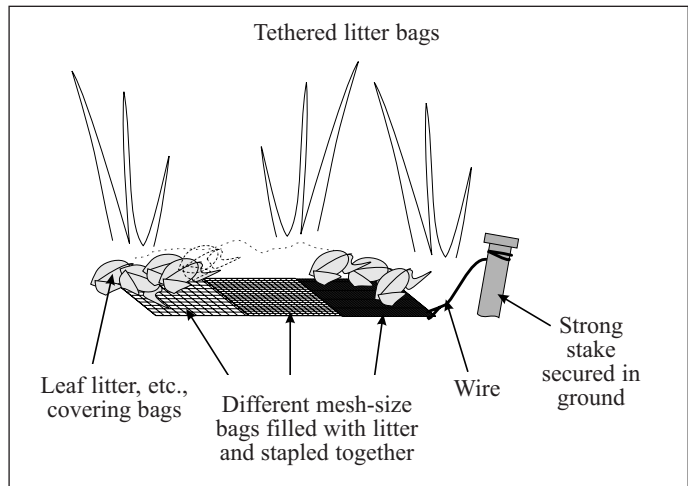
- Cut out and sew a number of plastic mesh bags leaving one end open (50 per mesh size; see inset). Mesh should be selected to fall near the ranges described in the section 'Litter Bags' (see chapter 8), i.e. approximately 4 mm, 600 μm and 10 μm .
- Collect freshly fallen leaf litter, if available, from an untreated area after ensuring that the vegetation type is representative of the treated area. Air or oven-dry leaves (60 °C) to constant weight before removing stalks and weighing 3 g dry weight portions of dry material into the mesh bags. (If prior warning of monitoring need is too short, fresh material will suffice but measure its dry weight later.) Fill at least 20 bags of each mesh size for each treated and untreated area and sew or staple the open end.

Method

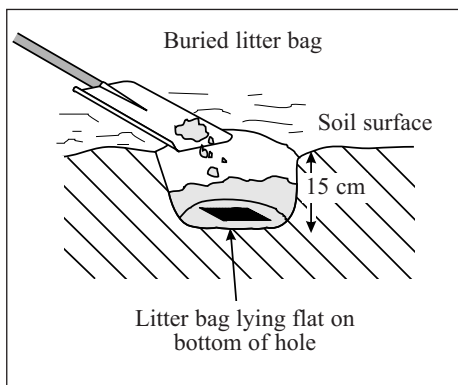
- Select five sites for the placement of bags in both treated and untreated areas, broadly matching the vegetation type between sites. Use random number tables to allocate sites and bury four bags of each mesh size horizontally in the soil at a depth of 1–3 cm at each site. Space the bags within a short distance of each other and not on top of each other (total of 120 bags). Replace the soil on top of the bags. Alternatively cut slits in the soil with a spade and insert bags vertically into the crevices, covering and tamping down the soil. Bury a few spares close by, but not in the same holes/crevices (to avoid disturbing others when dug up) to gauge the decomposition rate (see below).
- Carefully map the position of the bags in the soil as they are buried using a tape measure to measure distances from natural features (e.g. mark rocks or trees with paint). In open grasslands hammer in stakes or use piles of stones (but these may get removed); a GPS will be useful in finding these markers again.
- Leave litter bags in place from 2 to 3 months in cultivated and moist areas (or wet season) to 2 years in semi-arid environments. **Tip:** Remove a few of the spare litter bags at experimental intervals to gauge the rate of decomposition.



- At designated time locate the sampling site and dig a shallow trench around the area, leaving plenty of space around the buried bags to avoid damage by shovel or pick. Carefully remove the soil until the litter bags are located, remove bags from the soil (as quickly as possible if combining with a study of invertebrates) and place in a plastic bag, label and fasten using a knot or wire twist. Mark each plastic bag with site number, litter bag number, mesh size, collection date and also put a label, written in pencil, with the same information inside the bag.



- Process the litter bags as soon as possible. Rub off soil adhering to the outside of the litter bags then sun-dry the bags to constant weight (if in camp – a day in full sun) before re-bagging – do not confuse the labels! If returning to a laboratory, oven-dry at 60 °C to constant weight, then place the contents in a sieve (0.5 mm aperture) to separate the remaining organic material. Remove grass roots that may have grown into the bag and discard, shaking the remaining soil through the sieve rather than applying pressure, as fine organic debris is easily pushed through). Oven-dry the organic material retained by the sieve at 105 °C for 12 h to remove further moisture. Cool, desiccate and weigh the litter. Treat sun-dried litter in the same way.
- Subtract the dry weight of organic matter from the dry weight of the original material and express the difference as percentage degraded.



OTHER CONSIDERATIONS

If using large mesh bags (>10 μm) refer to section on litter bags in chapter 8 to accommodate the influence of invertebrate activity.