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INTRODUCTION

Many kinds of organisms live in the soil: algae, protozoa, fungi, bacteria, nematodes, worms, mites and a range of insects. Their composition, number and activity varies widely between biome and with seasons, which gives rise to marked changes during the climatic extremes of tropical and sub-tropical biomes. The algae have a production function in soil – carbon-fixation and in some cases, biological nitrogen-fixation – that is important in semi-arid soils and lowland rice fields. The majority of soil organisms live by consuming and decomposing organic material. In this way, soil organisms are responsible for maintaining the natural fertility of soils through processes that cycle plant nutrients and facilitate the flow of energy.

Pesticides can be applied directly to soils for the control of crop pests such as weeds, nematodes and insects or be deposited on soil as an indirect result of crop foliar or forest canopy spraying. A significant proportion (50% is not unusual) of chemical pesticides applied for the control of crop, forest, livestock and public health pests ends up in the soil, and their active ingredients put soil organisms and the processes that they perform at potential risk. Some processes accomplished by soil organisms are easily measured; often more easily than quantification of the actual organisms responsible. This can be very useful in pesticide impact assessment where a process, such as leaf litter breakdown, is studied rather than the complex range of organisms responsible for it.

Mineralization of organic matter, nitrogen transformations and biological nitrogen-fixation are key soil processes in both moist and dry tropical ecosystems. In naturally infertile ecosystems their role in maintaining productivity is prominent. The bacteria responsible for soil nitrification – the conversion of ammonium to nitrate – are slow to grow and are very sensitive to pesticides. Any inhibition of nitrification is indicative of stress in the dynamic equilibrium of nitrogen in the soil, and as nitrification is essentially an aerobic process occurring in the upper soil horizon where pesticides are held, the process is considered as key.

Biological nitrogen-fixation has a conspicuous role in the nitrogen economy of impoverished soils. Nitrogen-fixing algae and bacteria supplement soil nitrogen through the process of using (fixing) nitrogen in the air to make cell proteins that, on decay of the organisms, release the nitrogen into the soil. Nitrogen-fixing algae (Cyanobacteria) are common in flooded rice fields, shallow pools or as encrustations on soils, the bases of trees and under rocks. Herbicides and some insecticides are known to influence the growth and nitrogen-fixing activity of these organisms but current knowledge of such impacts in tropical regions is very limited. Pesticides may also affect nitrogen-fixing bacteria that are symbiotically associated with legumes (*Rhizobium* spp.) and free-living soil diazotrophes, but the fact that they are associated with the rhizosphere provides them with considerable protection from indirect contamination, although not from soil sterilants and nematocides. Algae are also useful in binding soils and protecting them from wind and splash erosion and thus help to increase soil stability.

Decomposition of crop residues and leaf litter results in the release of nutrients and energy that is critical for the maintenance of soil fertility and productivity in tropical ecosystems. The process is achieved through the

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cutting and shredding action of fauna and the subsequent breakdown and mineralization of organic matter by fungi and bacteria to chemical elements. Pesticide effects on soil micro-organisms and their activity are widely reported but generally short-lived in temperate climates. However, small deficits in soil nitrogen budgets can be expected to have greater impact in nitrogen-limited environments such as infertile savanna grasslands and woodlands. By using pesticides as a source of energy for growth, micro-organisms also play an important role in the breakdown of pesticides, some of which are more resistant to decomposition than others.

Breakdown of organic matter by soil organisms is accompanied by the uptake of oxygen and production of carbon dioxide as soil microbes and soil fauna respire. Soil respiration is a useful indicator of pesticide impact on organic matter breakdown. However, the fact that many types of microbe are involved in the process has the effect of reducing the sensitivity of respiration as an indicator, because microbes not affected by pesticides continue to metabolize. Respiration is readily measured in the field as carbon dioxide release.

In the process of consuming large quantities of soil to extract its nutritive value, earthworms are responsible for considerable soil mixing, breakdown of organic matter and release of nutrients that collectively help to maintain soil fertility. They are also an important part of the food chain for birds and represent a useful bioindicator of soil pesticide contamination and a measure of risk to their predators, especially birds. Although not present in all soils, the estimation of earthworm populations or their casting activity is an indirect way of detecting perturbations of soil processes. Termites frequently occupy a similar role in some tropical ecosystems where earthworms are absent (see also chapter 8).

Soil processes are affected by a number of environmental variables such as soil texture, moisture content and pH. Most soils have a pH in the range 4–8. Humid forest soils tend to be acid (pH 4–6) and semi-arid grassland soils neutral to alkaline (pH 7–8). In the context of this chapter, the importance of soil pH is in determining the solubility of soil minerals and nutrients that affect soil microbial processes and in influencing the toxicity of pesticides to soil organisms. Soil texture refers to the proportions by weight of sand, silt and clay that, together with organic matter content, influence water-holding capacity, leaching and nutrient storage capacity (see chapter 5). As these parameters not only control the dynamics of soil processes but also the availability, degradation rate and persistence of pesticides, the measurement of soil texture, moisture and pH is essential for the interpretation of pesticide impact on soil processes.

Other factors affecting toxicity, movement and persistence of pesticides in tropical soils are rainfall and leaching, sunlight, temperature and wind speed. For this reason a record of site meteorological conditions should be kept during monitoring periods (see chapter 5).

This chapter provides a selection of cost-effective methods for the measurement of key soil processes at risk from pesticide use and also some guidance on their selection, application and operation in a monitoring protocol.

Useful reference works on the impacts of pesticides on soil micro-organisms and soil microbial processes are Domsch *et al.* (1983) and Sommerville and Greaves (1987). A comprehensive collection of chemical and microbiological methods is provided by Weaver *et al.* (1994) and the *Tropical Soil Biology and Fertility* handbook (Anderson and Ingram, 1993), although these have demanding requirements for laboratory facilities and technical expertise. Doberski and Brodie (1991) have compiled a useful series of appropriate techniques for terrestrial habitats.

STUDY DESIGN

Let us assume the aim of the pesticide impact study is to identify biologically significant changes in soil processes that are attributable to the action of the pesticide. The strategy to achieve this requires planning to ensure that appropriate processes are monitored, and the techniques are feasible and applied in a way that allows valid statistical comparison of data. This section should help with those decisions.

Consider from what you know of the specific pesticide, dose, application method, the soil type and the likelihood of soil contamination just what processes may be affected. Aerial spraying is less likely to contaminate soil where vegetation is dense (e.g. forest) than in open woodland or grassland. Dusting, particularly in open and crop areas, is likely to contaminate soil. Remember that dose rates in crop areas are usually much heavier (10–100 more) than those used in less managed environments as, for example, with control of tsetse fly, locust and armyworm (but there are exceptions to this generalization). Table 7.1 should help reduce monitoring choices based on the relative sensitivity of organisms.

Table 7.1 Indicative soil populations and processes by pesticide and soil type

Pesticide type	Soil type	Indicative sensitivity	Useful methods
Fumigants/sterilants	All	All soil faunal and floral populations and processes	SN, SR, EP, SA, LB,
Fungicides	All	Fungal populations, symbiotic nitrogen-fixation	LB
Organochlorines	All	Earthworm populations, litter breakdown, nitrification, micro-arthropods	SN, EP, LB
Organophosphates	Sandy, low organic matter	Nitrification	SN, EP
Carbamates	n/a	None indicated at recommended doses	
Pyrethroids	Low organic matter	Nitrification	SN
Insect growth regulators	Sandy, infertile	Soil micro-arthropods	Chapter 8
Nematocides	All	Litter degradation, respiration, nitrification	SR, SN, LB
Herbicides	Infertile	Algal populations and nitrogen-fixation (fungal populations for atrazine)	SA
Phenyl pyrazoles (fipronil in particular)	All	Soil fertility through action on soil termites, litter breakdown	LB

SN = soil nitrification; SR = soil respiration; EP = earthworm populations; SA = soil algae; LB = litter bags.

Then consider the habitat sensitivity.

- Established agro-ecosystems are fairly robust but any use of persistent chemicals, e.g. organochlorines, nematocides and soil sterilants would indicate monitoring of all processes except biological nitrogen-fixation.
- Relatively unmanaged areas such as woodland and grassland savannas, sub-humid and humid forest are at relatively low risk from migrant pest control (locust, quelea, armyworm), grasshopper, forest pest and public health control measures, provided recommended dose rates are adhered to. For these categories of pests, recommended rates are frequently exceeded as an insurance speculation against the high costs of repeating operations in remote areas. Monitoring of nitrification is the minimum requirement; suspected overdosing requires, in addition, respiration and litter breakdown measurements.
- Despite the above, soils low in organic matter and natural fertility plus those in areas with short growing seasons are at greater risk; monitoring of nitrification, biological nitrogen-fixation and litter breakdown is indicated. Included in this category are the aridisols, ultisols, alfisols and oxisols.

- Land with conservation designations has a high political profile and may require monitoring in accordance with national requirements.

Soil and pesticide properties greatly influence the behaviour, availability and subsequent toxicity of pesticides to soil microbiota and their functions. Thus, pesticides applied to soils with low clay or organic matter content may initially be more biologically active because of diminished binding and adsorption on to organic and clay mineral particles, while a relatively volatile pesticide is likely to evaporate quickly from soil surfaces. Persistent pesticides might be expected to have longer-term effects on some microflora. So some generalizations about the risks to, and sensitivities of, populations and functions are possible but the relative scarcity of field and bioassay data makes predictions for aiding study design rather insecure.

Since the distribution of pesticides in soil is far from uniform and the natural variability of soil populations and processes is high, *in situ* monitoring of pesticide perturbations of soil functions is often impeded by unmanageable sample replication, particularly in uncultivated areas. A compromise is to prepare and deliberately expose native soil to the pesticides in areas where pesticide contamination is anticipated (between crop rows, under the path of helicopters, downwind of aerosol droplet generators, etc.) and then incubate them under field conditions. Methods for nitrification and respiration measurement are given to deal with these conditions.

Change in a population state or the rate of a soil process must be identified and then distinguished as either an outcome of pesticide use or natural variation. A sound experimental design is required at the outset of monitoring to achieve this goal or the data collected may not stand up to statistical scrutiny; chapter 2 is obligatory reading in this respect. It is equally important to select sample and replicate sites in control and treated areas that are matched in terms of soil and vegetation type. At the microhabitat level, for *in situ* assays such as respiration or worm casting activity, soil moisture, shade and ground cover should be compatible.

Microbial growth and activity are limited by soil water and temperature. Comparisons of microbially mediated processes must, therefore, take account of soil moisture. Any conclusions drawn from procedures used to establish the rate of any process measured at different moisture levels or temperature are invalid. Field methods for estimating soil moisture and water-holding capacity are provided in chapter 5. Make a habit of noting down the prevailing air and soil temperature, sun and shade at each study site.

In dry areas, soil activity may be arrested in the dry season with the result that some processes, such as litter breakdown, may take months to complete. The same process in the wet season may be completed within weeks. These are seasonal design considerations for the monitoring protocols, i.e. long time frames may be necessary and collection of pre-spray data (always recommended if affordable), may be impossible if phasing of spraying and post-spray monitoring crosses seasons. Under these circumstances, monitoring at the control (untreated) site will identify the natural variations of activity. The time-scale of post-spray sampling will depend upon the bioavailability and persistence of the pesticide in the soil. A 30-day period should suffice for *in situ* measurements of respiration. Dry season spraying (e.g. for tsetse fly control) will delay the retrieval period of litter bags and force the use of *in vitro* techniques for nitrification and respiration.

Maps (1:50000) and a four-wheel drive vehicle are prerequisites for locating and maintaining sampling sites in woodlands and grasslands in all seasons. It is not always advisable to sample just off a track: explore widely but within sensible limits and in reach of the camp or laboratory.

SAMPLING TECHNIQUES

Soil nitrification

This method indirectly measures pesticide impact on nitrifying organisms that are responsible for the transformation (oxidation) of ammonium to nitrite and nitrate. The criterion for the measure is any observed

delay in the build up of nitrate and is arrived at using a nitrate electrode to determine $\text{NO}_3\text{-N}$ in water-extracted soil samples. The electrode, which measures nitrate ions, is similar to a pH electrode in size and use. The field test is not performed *in situ* but on prepared soils amended with ammonium-N before exposure to the pesticide and subsequent incubation at field temperatures. The method sheet is designed for undertaking the whole, long-term procedure (40–50 days) in the field. However, the procedure can be started in the field and continued under standard incubation conditions in the laboratory if the establishment of a field laboratory is not practicable.

An estimate of dosage received by the soil is obtained from standard magnesium oxide slides (see Matthews, 2000) or water/oil-sensitive papers placed at ground level (see chapter 4). The most accurate but expensive procedure is to analyse the residue content in samples of exposed soil (see chapter 6); it is not practicable on a routine basis.

Limitations Ion-specific electrodes are fairly expensive and not particularly robust. A pH or millivolt meter is also required. The procedure is fairly demanding and requires attention to detail. The assistance of a chemist may be required.

Processing Simple aqueous soil extractions of nitrate. It is important to replenish soil water content and air inside the sample containers daily. Use deionized water if available for re-wetting and extraction of nitrate (0.25M K_2SO_4 may extract more from some soils).

Resulting data A graphic representation of nitrate concentration vs. time is a simple and effective way to show a depression of activity. Nitrate concentration is usually expressed as $\mu\text{g NO}_3\text{-N g dry weight soil}^{-1}$. The ecological importance of depressions in activity caused by pesticides is normally gauged against those observed under conditions of natural stress (e.g. drought or waterlogging). A 90% depression of nitrification for up to 30 days may be regarded as not ecologically significant. Longer periods, particularly in semi-arid climates, may not be tolerable, as the seasonal activity is restricted by rainfall.

Sampling period Sampling period depends upon temperature and moisture but 2 months would be a useful average to use for planning.

Equipment Nitrate electrode, reference electrode, millivolt meter or pH meter.

Staff required 1.

Biological nitrogen-fixation

Field methods for the indirect measurement of biological nitrogen-fixation are available (Holfeld *et al.*, 1979; Grant, 1986, 1988), but the difficulties of accessing portable gas chromatographs and clean acetylene in many tropical countries restrict measurements. Yatazawa *et al.* (1984) provide plans to build a suitable portable gas chromatograph. Alternatively, gas samples in vacutainers may be brought back to the laboratory for measurement on a gas chromatograph. It is recommended that the advice and support of a local soil microbiology/agronomy laboratory is sought for planning acetylene reduction assays. No methods sheets are provided as specialist assistance will be required and the reader is recommended to see Robertson *et al.* (1999) for field techniques for soil and Grant (1986) for water.

Soil respiration

Plant roots, soil macro- and micro-fauna and the soil microbial biomass all contribute to soil respiration. The *in situ* measurement of changes in respiration rates are, therefore, not as straightforward to interpret as using dug soils, which may be prepared in a way to standardize some of these variables. *In situ* respiration techniques trap or continuously measure carbon dioxide released from an enclosed area of soil so care must be taken to match the vegetation types and their spatial distribution between areas compared, and to sample between plant stands. It is also advisable to dig up the soil below an enclosure, after measurement, to measure the percentage soil moisture (see method sheet) and to gauge the extent of any root mass or earthworm populations that might skew respiration rates between sites. The most appropriate time to measure respiration *in situ* is when soils are moist or wet, as there is little microbial activity and respiration in the dry season.

The use of prepared soils largely overcomes the difficulties of root and invertebrate contributions to soil respiration, and because soil moisture significantly affects respiration, this too may be standardized. Soils are pushed through a sieve to remove roots and macro-invertebrates before being amended with organic matter (if necessary) and water. They are subsequently exposed *in situ* to pesticide and monitored over a period of 30–40 days or more (tropical conditions). The organic matter amendment may be local dried grass either pushed through or milled to pass a 0.5 mm sieve.

Limitations *In situ* respiration is most conveniently measured with a portable infra-red gas analyser (e.g. Grant, 1990), but the capital cost is high. Draeger tubes are a less expensive alternative but their availability may be restricted. A classic titrimetric method for long-term *in situ* rates involving carbon dioxide absorption by alkali is, therefore, provided (attributable to Anderson, 1982). *In vitro* (prepared soils) estimates of respiration provide standardized test conditions and a powerful tool for comparing pesticide impacts but respiration rates cannot be translated to field rates. The equipment necessary is relatively inexpensive and robust but the gas analysis tubes are not reusable.

Resulting data Graphic representation of the rates of respiration expressed in relation to either soil dry weight or area is useful to determine and demonstrate any depression of respiratory activity related to pesticides. Examples of outputs may be ml CO₂ g dry weight soil⁻¹ h⁻¹ or mg CO₂ m⁻² h⁻¹. Plotting the soil temperature and moisture on the same graph is also helpful in assessing causes of rates of change in respiration as small fluctuations in either greatly affect microbial activity. Follow the guidelines given under the *Soil Nitrification* section for an interpretation of the ecological significance of depressions of respiration. The use of *in vitro* techniques during the dry season (i.e. on dry soils) is questionable, because if soils are not wetted to stimulate activity, pesticides would be denatured or dissipated by UV light, heat and volatilization, reducing their toxicity by the time it rained. However, spraying normally accompanies vegetation growth after rains: tsetse fly control is the exception as tsetse feed on animals.

Sampling period Typically up to 1 month or more, sampling CO₂ at about six intervals during the month. *In vitro* techniques can be used in any season – *in situ* sampling may be limited to the wet season. Soils in some regions may remain moist enough in the dry season to support microbial activity. *In situ* measurements are made before and after spraying or soil treatment.

Equipment Infra-red gas analyser or Draeger tubes, or simple titration glassware and reagents.

Staff required 1.

Soil texture, moisture and water-holding capacity

See chapter 5 for methods and discussion.

Earthworm populations and activity

Methods for estimating relative earthworm abundance in soils are simple and robust, relying on either hand searching of dug soil or applying an irritant to the soil that causes earthworms to surface, where they are collected or counted. Hand sorting of worms from soil, although tedious, is generally more effective than the drench technique that uses irritants such as formalin and detergent. Both methods rely on marking out sites in treated and untreated areas to either dig/core sample or drench with irritant. Coring and digging small pits are convenient as soils can be transported to a laboratory for hand sorting while drenching requires staying in the field for a day or more.

Limitations Earthworm distribution is affected by soil conditions, moisture, organic matter content and a number of replicate samples may be needed to estimate populations. It is important to match soil type and texture in areas chosen for sprayed/unsprayed monitoring. In the dry season, more drench (irritant) needs to be applied to expel the worms from deeper layers.

Processing Easy but tedious sorting of soils by eye using forceps. Using a drench, worm collections are easy but vegetation can obscure surfacing worms and the irritants can affect human skin (especially formalin) so nitrile/rubber gloves must be worn (see chapter 3). The taxonomy of worms is a job for a specialist – at least at the outset.

Sampling period Estimate earthworm populations every 10–14 days but using a different transect across the area each time as the drench is persistent and will affect the behaviour of the worms.

Equipment Soil corer, trowel or spade.

Staff required Heavy fieldwork is best done with 2 people.

Earthworm activity (feeding and burrowing) is determined by counting surface worm casts or recording the rate of casting. Some casts are distinctive enough to separate one species from another, increasing the information value of the technique. Specialist taxonomic help will be needed initially. The techniques described are based on observations and counts of casting activity at random points along a transect or inside quadrats thrown in treated and untreated areas. Remember to note the weather at the time of sampling and determine the soil pH and percentage soil moisture, as these parameters affect the distribution of worms.

Limitations Not all soils will contain earthworms at high enough densities to count or observe casting, but in moist soils that contain sufficient organic matter as food, the techniques are reliable. Wet season rain can destroy casts.

Processing None.

Resulting data Numerical counts of casting, perhaps by species.

Sampling period The counts are made at intervals of 2 days to 1 week in the wet season. Activity will be curtailed or reduced in the dry season (unless irrigated). Pre- and post-spray estimates should be made of both earthworm populations and activity.

Equipment No special equipment required.

Staff required 1, but 2 people will speed up the layout of transects.

Old but useful general references for earthworm biology and population estimation are Madge (1969) and Edwards and Lofty (1972).

(For methods to determine the abundance/activity of other soil invertebrates, see chapter 8.)

Litter bags

Litter bags are used to gauge the rate of organic matter (leaf or root litter) decomposition in or on the surface of soils by the soil decomposer community. As decomposition is effected by the soil fauna, microflora and associated soil enzymes, their relative contributions are crudely distinguished by burying a known weight of litter in bags of differing size mesh, which restricts the size of organisms that can enter the bag. After a period of time in the ground, from a few months to 2 years – depending upon decomposer activity – the residue is weighed. Use of the bags to determine the invertebrate decomposer role is described in chapter 8. Microbial action is gauged from the use of very small aperture mesh (10–60 μm) but remember microbes also contribute to breakdown in the bags designed to exclude invertebrate groups (e.g. 600 μm , 1 mm and 4 mm meshes). In these larger mesh bags, invertebrates chew up organic matter leaving it open to accelerated breakdown by micro-organisms. Fallen leaves provide an ideal source of litter. Litter should be air or oven-dried (60 °C) before being buried because variations in litter moisture affect initial decomposition rates.

Limitations A lot of bags, perhaps 250 or more, are required to estimate litter processing in a quantitative fashion and their production is labour-intensive. Once buried, they can be hard to locate and so detailed site maps, markers and photographs are necessary. A hand-held global positioning system (GPS) is useful for locating markers in remote areas. Very fine mesh bags can trap air, resisting its displacement with water for a short period. Litter bags exposed on the surface of the soil should be tethered to reduce the likelihood of being washed away in storms or being removed by wildlife. Fine mesh can be holed by termites and ants. Nylon mesh can be difficult to source in some countries and is time consuming to sew.

Processing Care must be taken not to lose bag contents when removing bags from the soil (some bags may be holed). Bags placed vertically in the soil are less vulnerable to losses at removal. Sieves are needed to separate soil particles from remaining debris; it is inevitable that some organic matter will be lost. Reducing the period of burial can reduce these losses.

Resulting data Dry weights of litter remaining or percentage loss can be graphically represented for bags buried in treated and untreated areas.

Sampling period This depends upon the distribution of rainfall and soil type. One wet season of perhaps 3 months or more should be sufficient – a single dry season may not be sufficient as soil microbial activity is negligible.

Equipment Spade, trowel, wire, tape measure and litter bags.

Staff required 2.

Soil algae

In the wet season or shortly after rains, the presence of algal growth on soils is obvious from the soil's green colour. On sandy soils they are less obvious as soil moisture loss is rapid and the algae may appear much darker and encrusted. Any uncertainty can be resolved by wetting a sample for a day or more and smearing a microscope slide with a thin layer of the soil sample before examining it under a high power microscope. The help of a soil microbiologist or phycologist will be necessary if you have never closely observed algae before. A simple belt quadrat technique to assess algal cover is described which only requires the estimation of algal cover within a quadrat placed at intervals along several transect lines.

Limitations The only real limitations of the belt quadrat technique are statistical, and concern the need for random stratified sampling of an area (see chapter 2).

Processing No processing is required as the method is based on visual observation.

Resulting data The quadrat data are used to determine algal cover and a histogram or area diagram can be used to present it.

Sampling period The impacts of herbicides or insecticides on algal grazers should be evident using weekly samples over the period of a month.

Equipment A compound microscope to confirm the presence (and species if interested in taxonomy) of algae, quadrat, string and tape measure.

Staff required 1.

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