

Preamble

I am posting the following notes, which are adapted from a discussion on soil macrofauna sampling which took place last June at IRD Paris, under the auspices of the IBOY Macrofauna Project. This project is basically a data collation exercise and does not involve new sampling, but is concerned with standardizing methods. The discussion was about monoliths, but one can substitute the term “litterbags” and the issues are mostly the same: How many? How placed? When placed? Where placed? How replicated?

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1. Terminology used in this discussion:

REGION – geographical, geopolitical or biogeographical demarcation (large).

LOCALITY – a site (generally a larger site) or a series of adjacent sites.

ZONE – A plot or a series of plots with a characteristic original vegetation, soil and climate. A zone can be characterized by several land-uses. A zone can be sampled via a plot or several plots, and at different times or seasons. In context of GLIDE, same as BIOME.

LAND-USE – the current management, including time since last felling or thinning for forests.

PLOT – a piece of land where litterbags are placed. (PARCEL – the same as a plot OR a unit of vegetation management on a land-use map. SITE – a place where you do fieldwork, generally the same as plot).

2. Sampling strategy: what is the basic approach and is it valid?

The basic choice for litterbag placement seems to be a) along a transect of some kind at some agreed absolute or relative spacing or b) in a block arrangement of agreed dimensions and litterbag density. Broadly, transects are a better way of dealing with horizontal (= patch) heterogeneity, while blocks provide a better framework for applying treatments (such as naphthalene) and estimating point variance. There also seems to be some historical divergence between tropical (transect-based) and non-tropical (block-based) work.

Many approaches to sampling tropical soil biotas have evolved from the basic field transect. A transect of 40 x 4 m is recommended for macrofauna by Anderson and Ingram (1993). The main premises are to have rapid assessment (a manageable number of samples to collect and sort/extract with few field staff), to be able to address as many taxonomic groups as possible at the same time and in the same plot, and also to be able to

deal with the small parcels of fallow and food-crop field which typify tropical subsistence agriculture. The TSBF transect was designed for the sampling of other soil biota and above-ground vegetation, in addition to macrofauna; consequently the spatial scale of the transect is small in relation to the known horizontal heterogeneity of many macrofaunal groups. There may therefore be a case for extending the transect to more than 40 m, where plot size and resources permit. However, in all circumstances the minimum transect length should be 40 m, even if this means breaking the transect into parallel fragments.

A review of the use of the TSBF transect by Alternatives to Slash and Burn Programme (ASB) is given in Bignell et al. (2001), due out later this year (but am happy to supply electronic preprints: contact me at d.bignell@cwcom.net). Should not a scheme for the standardized monitoring of litter decomposition follow a standardized scheme for sampling soil biota, i.e. we use a TSBF-style transect and place the litterbags along it, more or less in the same way one might select intervals for digging monoliths or coring the soil?

All regularly spaced samples on a transect are autocorrelated to some extent, and therefore do not constitute independent samples. This would also apply to block designs to a large extent, but is more difficult to test mathematically. Optimum spacing is likely to differ from one taxon to another and will also vary with soil type, and while statistical procedures to determine the extent of autocorrelation exist, more than 50 monoliths (=litterbags) per plot would be required to employ them and get a definitive answer. There is probably a case for commissioning a calibration study on the efficiency of transect/litterbag sampling, preferably in selected, relatively diverse zones (or where resources for such work, say by Honours project students, are available). In such a study, transect sampling at varying transect lengths and litterbag separations would be compared with exhaustively sampled actual assemblages and populations. Optimum litterbag separations could also be determined for major taxa. Such studies appear to be absent from the literature, but could provide validation for the methods we eventually adopt. Otherwise, we have only historical and logistical reasons for our choice, rather than a *a priori* scientific principle.

3. Replication

The basic unit of quantitative data collection from monoliths is the mean abundance or density of organisms (nos m⁻²) per treatment per transect or per block, averaged across all the monoliths (i.e. one transect or one block is a single sample). Can this concept be transferred to litterbags, for example by using organisms per unit of litter sampled per time period, averaged across the transect or block? If sampling records retain details of data from individual bags, the variance associated with litterbag sampling (not the same thing as the variance of mean abundance) can be recorded. It may be useful to report such variance, for example as the 95% confidence intervals associated with a (back-transformed) geometric mean (see Eggleton et al., 1996). However, it should be noted that log transformations do not necessarily normalize abundance and biomass datasets, either within individual transects/blocks or between plots with the same treatment.

The basic unit of qualitative sampling is the occurrence (presence/absence) of nominated taxonomic groups, although this may be combined with abundance data to generate diversity indices. Presence/absence also suffers from possible autocorrelation, but at least avoids the difficulties with variance generated by the clumped distribution of so many soil organisms. In termite work we have used the concept of relative abundance, i.e. we sample along a 100 m transect divided into 5 m sections: relative abundance is the number of transect sections (maximum 20) in which a particular species occurs. Some journal referees accept this and some don't (20 sections is enough to estimate mathematically the extent of autocorrelation and to construct a species/area curve).

It is important to sample more than one plot per land-use in order to have genuine replication. This is really more important than the transect vs block argument. 3-5 is the optimum number, although this may not be feasible in some localities. Where plot numbers are small, it may be better to reduce the number of treatments (or amalgamate treatments) rather than increase the number of transects/blocks per plot, which does not facilitate tests of statistical differences between treatments based on the comparison of variances. Therefore in the scheme of litterbags being proposed (6 per zone per time period), two separate plots would receive 9 bags each. Would this also help protect against disturbance?

4. Suggestions for additional sampling

For teams that have the time and resources, additional qualitative sampling could be carried out on the transect or within blocks by a) the investigation of microsites within a 2.5 m radius of each litterbag and b) by extracting the litter from 1 x 1 m quadrats in Winkler bags. Microsite investigation should be time-limited; 20-30 minutes per search (for two persons) is suggested as appropriate. Winkler bags are air-dried under cover from rain, a process which extracts ants and beetles efficiently. However the number of samples extracted per transect/block will depend on the number of bags and the drying facilities available. 3-4 samples per transect are suggested. One sample (i.e. litter from one quadrat) will typically require two Winkler bags to dry. There may be merit in additional qualitative sampling off transect, for example by the use of pitfall traps and by investigating termite and ant mound-nests in an area of approximately 40 x 30 m adjacent to the transect/block. The arguments in favour of pitfalls are that they sample mobile forms such as ground beetles and spiders, which might otherwise escape during litterbag recovery; the arguments against are that they are biased in favour of such fauna and are non-quantitative. Pitfall traps are also subject to disturbance (by insectivorous mammals and reptiles looking for a free lunch!).

5. Special methods for social insects

Special measures for social insects are proposed to take account of their high abundance (huge abundance in the tropics) and marked patchiness: a nest could contain millions of individuals, none of which might be sampled from litterbags in a short transect located some distance away, and the contribution of the species concerned to a macrofaunal

assemblage could thus be completely missed. On the other hand, a highly populous nest sampled directly by a nearby litterbag could produce a large overestimate of overall numerical or biomass density. In general, the TSBF transect is recommended to be placed so as to avoid direct contact with termite and ant nests. For discussions, see Eggleton and Bignell (1995) and Eggleton et al. (1996). The protocol for a 100 x 2 m transect designed to assess termite biodiversity (and feeding group representation) is given by Jones and Eggleton (2000). In suitable circumstances this can also be deployed in parallel with the TSBF transect.

This is really an argument, similar to 4. above, for additional qualitative microsite sampling to establish the dominance, or otherwise, of social insects. Anyone who has ever worked in the tropics and sub-tropics will understand this point.

6. Note biogenic structures

This basically means a) noting the presence of earthworm casts and/or termite sheeting within some specified radius (e.g. 2.5 m) of the litterbag and b) trying to make some assessment (presence/absence) of soil faunal faecal pellets present in material sampled from the litterbags. In its qualitative form, this is a fairly simple “add-on” to the project which can generate important information (and especially if litterbags are designed to exclude earthworms).

Protocols for quantitative determination in forests are given by Bernier and Ponge (1994), Bernier, (1996; 1998), and Ponge (1999). Quantification of soil sections is discussed by Bal (1970) and Jongerius (1963). For agricultural land, modified protocols are given by Topoliantz et al. (2000). This assessment demonstrates the amount of soil processed by ecosystem engineers. The method consists in cutting off a soil block (5 to 8 cm in section and 10 to 15 cm in depth depending on the soil type) with a sharp knife. Each profile is divided in visually homogeneous layers 0.5 to 2 cm thick which are immediately fixed in 90% ethanol. The soil layers are separately spread in Petri dishes filled with 90% ethanol then observed under a dissecting microscope at x40 magnification. The components of the soil matrix are quantified by a point-count method using a transparent grid laid upon the soil layer in alcohol. We obtain the percentage in volume of each component by dividing the number of points above it by the total number of points above solid matter. Concurrently, faeces are identified from soil animal cultures (diplopods, isopods, enchytraeids...).

The message here is that decomposition cannot be assessed independently of processes-generating organisms (one of the drawbacks of the litterbag approach), so a list of species extracted from litter + a list of major players in the soil system on which the bags are sitting is better than just a list of what emerges from the Tullgren funnels.

7. Sampling tactics: when, where and how?

In tropical systems, it is usually recommended that sampling takes place at the end of the rainy season ; and elsewhere, when accessible biodiversity is thought to be greatest.

Large seasonal differences may occur in species diversity, relative abundance and vertical distribution. Ideally, sampling should be repeated in the same plot under contrasting conditions (for example during both wet and dry conditions). However, there is no point in sampling under severe conditions when faunal activity is nil (extreme drought or cold), just for the sake of having co-ordinated sampling in all sites.

8. Stratified sampling: dealing with horizontal heterogeneity.

Stratified sampling may be appropriate to a major topographical or vegetational feature of a zone or plot, or where the distribution of macrofauna is expected to be highly clumped. This means departing from the basic transect or block scheme and either:

a) marking a large sampling area (say 40 x 40 m, which incorporates most of the obvious heterogeneity of the site), then allocating the litterbags to 1 x 1 m (or 2 x 2 m) quadrats selected at random within it. The quadrats are sampled qualitatively or quantitatively for soil animals at the same time as the litterbags are monitored for decomposition rate and chemistry.

or b) breaking a standard transect into 2 or more sections which are placed to run (separately) through the main vegetational or topographical features of the site.

Protocols cannot be prescriptive on the positioning of transects, or the size of sampling areas (or block sizes), owing to differences in plot size and shape (and therefore edge effects), and to variations in vegetational patterns, especially clumping of grasses and trees. Little or no guidance exists in the literature, other than that transects should include the major plot heterogeneities of interest (for example upper and lower slopes, under trees and between trees, in tussocks and between tussocks, in crop rows and between crop rows, etc.) and should avoid features which are likely to contain none of the organisms being addressed (steep gullies or cliffs, streams, waterlogged ground, fresh skid trails etc.)

One advantage of the transect over the block, is that the transect can incorporate other natural features of the site which seems likely to contribute to its biological heterogeneity, such as dips, dry stream gullies or small canopy gaps. Some subjective judgement is often necessary to decide on the most suitable line, especially where the treatment plots concerned are small. Transect lines do not need to be absolutely linear, and can be turned through angles of up to 90° to avoid natural obstacles, for example large tree trunks, as long as they do not re-intersect with themselves. In small plots a transect can be turned, successively, through two 90° angles to run back towards the starting point, but the two main "arms" of the transect should be at least 20m distant. Alternatively, two half transects can be run in parallel. It is advisable to make a note of the starting point, initial compass bearing of the transect line and any major directional changes. If two 40 m transects are employed for replicated sampling, other qualitative sampling such as a 100 m termite transect should run between them, but allowing sufficient space to avoid mutual interference. Perhaps similar arguments apply to block size and the mutual separation of litterbags within it.

Stratified sampling might include the division of a transect into two 20 m halves, one placed in a particular type of vegetation patch and the other in a second, or might include the special sampling of an under-tree soil on or off the transect, combined with a conventional row of litterbags between trees. In more complex versions, especially those designed for social insects, the soil might be sampled by transect litterbags while large woody items and mound-nests on or off the transect are considered as special cases and directly sampled to collect all associated animals.

In summary, it is important to make a subjective assessment of the scale of heterogeneity in any plot. Heterogeneity on the centimetre or metre scale can be accommodated by the basic transect or block scheme, but heterogeneity in units greater than ca. 10 m, or on a landscape scale, may require the introduction of a stratified sampling regime.

a) Equipment.

The following items of basic equipment are recommended. The list is useful to prepare the inexperienced for what may await them.

Tapes measures of 30 m and 10 m. machette (cutlass or parang), spade, plastic weave produce sacks for spreading on the ground, large diameter plastic or metal bowls (cuvettes), trowel, small plastic trays, fine forceps (or entomological forceps), fine paint brushes, sample vials in various sizes with secure alcohol-tight caps, Indian-ink pen (waterproof), stiff card for labels, notebook, ranging compass, large strong plastic bags (sealable), table and plastic chairs (for sorting), tarpaulin (for protection from heavy rain).

Both 80% alcohol and 4% formalin should be available for preservation of specimens (4% formalin is more suitable for earthworms and gastropods, which can be transferred to alcohol after about 4 days fixation).

3. Sorting and extraction

Hand sorting of litter samples for macrofauna (especially for ants which escape easily) may be a useful preliminary to Tullgren extraction. Hand sorting is widely assumed to be subject to error and inefficiency, but this is likely to vary between season and taxonomic group and with body size and soil type. Field assistants should collect all taxa (i.e. not specialize), although ants should be picked out first, as they have a tendency to escape.

Handsorting and wet-sieving might be useful qualitative checks on the effectiveness of dry heat extraction. Berlese or Tullgren-style funnels can be quite easily fabricated. Small quantities of litter or spent soil are dried slowly for 24 hours, using a low-wattage electric light bulb. Where electricity is not available, material can be dried under black plastic sheets in direct sunlight, but the mesh and funnel must still be in place to collect specimens. Wet sieving consists of brushing soil, with water, through a series of sieves of diminishing mesh size. Coarse sieves can remove most stones, roots and other plant debris, after which macrofauna can be intercepted by a final sieve of ca 1 mm mesh.

It seems most unlikely that Tullgren rigs will be standardized, but it should be possible to standardize the drying process to a specified water content. Each rig can then be calibrated at this standard to determine the drying time under ambient heating arrangements.

5. Biomass determinations

I suggest the use of fresh weight, measured in the field. Failing this blotted weight, after preservation, may be substituted. Other methods are reported in the literature, for example fresh weight after blotting, dry weight at 60°C overnight, drying to constant weight at higher temperatures, degutted fresh weight, degutted dry weight, fresh weight x a constant (for assumed water content) and head width (referenced to a calibration curve). However, we argue these have less biological meaning than fresh weight. As a minimum, the method of determination must always be specified.

6. References

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