

# Towards a universal sampling protocol for soil biotas in the humid tropics

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**Abstract** – This paper reviews the methods for the inventory of below-ground biotas in the humid tropics, to document the (hypothesized) loss of soil biodiversity associated with deforestation and agricultural intensification at forest margins. The biotas were grouped into eight categories, each of which corresponded to a major functional group considered important or essential to soil function. An accurate inventory of soil organisms can assist in ecosystem management and help sustain agricultural production. The advantages and disadvantages of transect-based and grid-based sampling methods are discussed, illustrated by published protocols ranging from the original “TSBF transect”, through versions developed for the alternatives to Slash-and-Burn Project (ASB) to the final schemes (with variants) adopted by the Conservation and Sustainable Management of Below-ground Biodiversity Project (CSM-BGBD). Consideration is given to the place and importance of replication in below-ground biological sampling and it is argued that the new sampling protocols are inclusive, i.e. designed to sample all eight biotic groups in the same field exercise; spatially scaled, i.e. provide biodiversity data at site, locality, landscape and regional levels, and link the data to land use and land cover; and statistically robust, as shown by a partial randomization of plot locations for sampling.

**Index terms:** agricultural intensification, deforestation, functional groups, sampling replication, sampling schemes, soil biodiversity.

## Em direção a um protocolo universal de amostragem de biotas do solo nos trópicos úmidos

**Resumo** – Este trabalho faz uma revisão dos métodos de inventariado da biota edáfica nos trópicos úmidos para documentar a (hipotética) perda de biodiversidade do solo associada ao desmatamento e à intensificação agrícola nas margens de florestas. A biota foi agrupada em oito categorias, cada uma correspondente a um grande grupo funcional considerado importante ou essencial para a função do solo. Um inventário cuidadoso dos organismos do solo pode auxiliar a gestão de ecossistemas e a sustentabilidade da produção agrícola. As vantagens e desvantagens de métodos de amostragem baseados em transectos ou em malhas são discutidas e ilustradas por protocolos publicados, desde o original “transecto TSBF”, passando por versões desenvolvidas para o projeto “Alternatives to Slash-and-Burn” (ASB), até o esquema final (com suas variantes) adotado pelo projeto “Conservation and Sustainable Management of Below-ground Biodiversity” (CSM-BGBD). Considerou-se a importância de repetição de amostras e argumenta-se que os novos protocolos de amostragem são: inclusivos, ou seja, desenhados para amostrar os oito grupos bióticos ao mesmo tempo no campo; dimensionados espacialmente, pois fornecem dados de biodiversidade nos níveis de lugar, localidade, paisagem e região e associam esses dados ao uso e cobertura do solo; e estatisticamente robustos, como evidenciado por uma aleatorização parcial das localizações dos lotes de amostragem.

**Termos para indexação:** intensificação agrícola, desmatamento, grupos funcionais, repetição da amostragem, esquemas de amostragem, biodiversidade do solo.

### Introduction

Soil is the habitat of a diverse array of organisms which contribute to the maintenance and productivity of ecosystems (Brussaard et al., 1997; Lavelle et al., 1997). Large numbers of farmers in the tropics have limited access to inputs of pesticide and fertilizer, but are forced to reduce the complexity of their agroecosystems in an attempt to increase production (agricultural

intensification). This results in obvious and generally unsustainable disturbances such as deforestation, loss of crop and background biodiversity, pollution, soil erosion, depletion of fertility and, ultimately, global environmental change (Gillison et al., 2003; Bignell et al., 2005; Moreira et al., 2008).

To understand the effects of different disturbances at the level of the soil biota, there is a need for standard methods and practical instruction for the inventory

of below-ground biodiversity that will allow the comparison of inventory results from different tropical countries and different benchmarks (i.e. experimental sites with fully defined physical and edaphic characteristics) on a valid scientific and statistical basis. This information and knowledge can then be used to better manage and conserve below-ground biodiversity (BGBD) in tropical agricultural landscapes, to maintain agricultural productivity and reduce the further extension of agriculture into pristine natural habitats.

Here, I summarize sampling and laboratory assessment methods for the biodiversity of a range of key functional groups of soil biota. This is a further elaboration and updating of methods and protocols that were initially assembled and drafted by scientists affiliated with the Tropical Soil Biology and Fertility Institute of Centro Internacional de Agricultura Tropical (CIAT), the EU-funded Macrofauna Network, the Natural Environment Research Council (UK)-funded Terrestrial Initiative in Global Environmental Research (TIGER), and in particular, the United Nations Development Program/Global Environment Facility-funded Alternatives to Slash-and-Burn Project (ASB). Methods for some functional groups of soil organisms were included in a pioneering handbook of methods by Anderson & Ingram (1993). Methods for the assessment of soil biodiversity are elaborated further in the ASB Lecture Note 6B edited by Swift & Bignell (2001), which also introduced the principle of functional groups in the inventory of soil organisms (see also Bignell et al., 2005). Independently, an authoritative manual of techniques for soil organisms and organisms inhabiting freshwater and marine sediments was published in 1996, edited by G. S. Hall, developed as part of UNESCO's contribution to the DIVERSITAS programme. Also, many relevant sampling issues are discussed in detail in Leather (2005), although the focus of this book is not specifically tropical, and most recently in Moreira et al. (2008).

Bignell et al. (2005) listed the five principal functions of soil biota as decomposition of organic matter, nutrient cycling, bioturbation, suppression of soil-borne diseases and pests, and environmental services (for example: watershed protection, preservation of soil structure, mitigation of greenhouse gas emissions and bioremediation after pollution events). A functional group was therefore defined as an assemblage of species, of any taxonomic affiliation and living at

whatever spatial scale, whose collective impact in a soil ecosystem is one of these generic functions, with the assumption that all five must be manifested in any soil that has sustainable fertility and structural stability. This sets the agenda for below-ground biodiversity projects focused on land use change and agricultural intensification, by specifying the groups of organisms that must be sampled or assessed together, but also raises the question of what relationship exists between species diversity, functional diversity (the number of functional groups), functional composition (the nature of functional groups) and the occurrence and intensity of ecological processes. While the functional group concept is, in one sense, a complication in soil biology (as it requires attention to be paid to both microfauna and microorganisms, as well as the more tractable larger animals), in another sense it is a convenience which circumvents the poor state of taxonomy for some groups and the lack of agreed or adequate methods for extracting and enumerating others. A further concern is the ecological impact of plants that affect soil, including vegetation cover determining soil climate, root penetration and water infiltration influencing soil structure and root exudates and litter quality mediating the supply of nutrients to soil organisms.

The global ASB programme, which looked for optimal schemes of tropical forest-based subsistence agriculture that would increase food security and retain environmental resilience (Kenya, 1997; Palm et al., 2005), addressed the effects of land-use change on soil biodiversity by designing field sampling to test the following hypotheses: A, agricultural intensification reduces soil biodiversity; B, Reduction in soil biodiversity compromises ecosystem functions needed for sustained productivity; C, above-ground and below-ground biodiversity are interdependent across scales of resolution from individual plant communities to the landscape; D, agricultural diversification promotes soil biodiversity and enhances sustained productivity; E, sustainable agricultural production at tropical forest margins is improved by enhancement of soil biodiversity.

These propositions have been at the heart of all tropical soil biological research to alleviate poverty for at least three decades, and will remain so for the foreseeable future, but meanwhile much progress has been made with the concepts of field sampling schemes, which is summarized here.

### Where are we now?

An early protocol for assessing soil biota in a concerted way was presented by Anderson & Ingram (1993). They proposed a system of five to ten standard monoliths, each of 25x25x30 (depth) cm arranged along a short line in each land use for soil fauna, and, as practiced, this seems to have evolved into a standard transect of 40x5 m, in which the monoliths are spaced at intervals ranging between 4 and 8 m. Other sampling methods for mycorrhizally infected roots, viable rhizosphere bacteria, viable rhizobia (i.e. root nodules) and soil physics and chemistry were also recommended, but spatial schemes and patterns for the field were not specified. The monolith is established by rapid trenching around its perimeter, then dissected from the top downwards in 10 cm slices. The monolith may seem something of a blunt tool, but if properly executed is effective for up to 13 major taxa of arthropod and annelid macrofauna, as it minimizes losses by escape. If the dissection is done thoroughly, it is also possible to obtain data on abundance and biomass, although the application of descriptive statistics to the results is problematical (see below). Further, the outer side of the trench is a good place to take soil samples for moisture physics and chemistry, including bulk density, a key consideration for soil animals. The main disadvantage of the monolith is the time required to sort the animals from the soil, which can only be done manually and should be carried out on site, because of the bulk of material and to prevent death of specimens during transportation (although evidence of the latter is anecdotal). For further discussion see Rossi et al. (2006) and Smith et al. (2008).

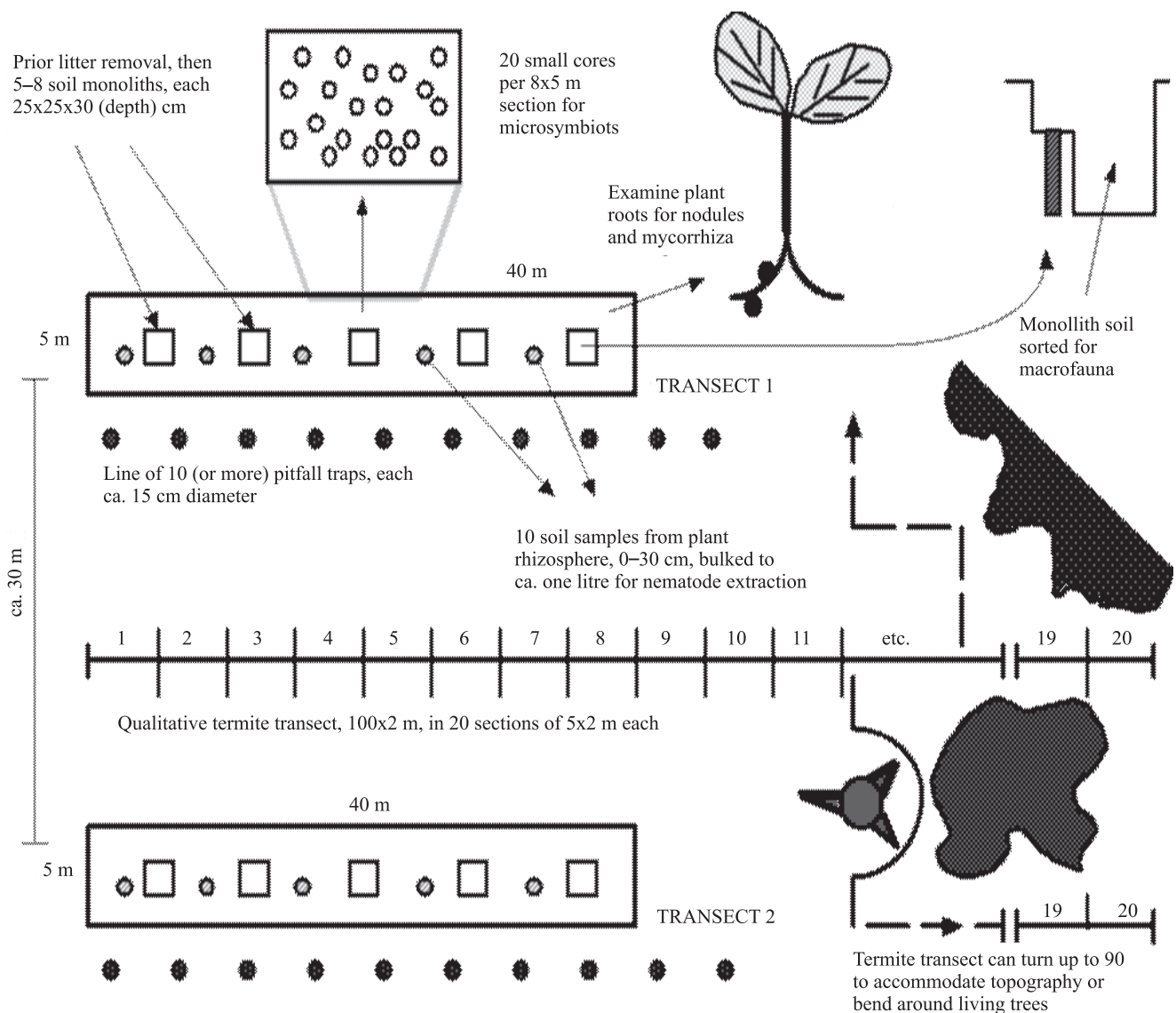
A more complex scheme was formulated by Swift & Bignell (2001), arising out of the experience of ASB sampling, which had run over the preceding decade (Figure 1). This also made use of the short transect with at least five monoliths, but in addition to a duplication of the transect there are also cores (subsequently bulked) distributed over the transect for nematodes and microsymbionts, inspection of roots for mycorrhizal infection and rhizobial nodulation (with subsequent laboratory analyses), parallel long transects for termite sampling (Jones & Eggleton, 2000) and the alignment of pitfall traps with the long transect to collect more of the soil fauna. This represents very intensive sampling within a single plot, amounting to 290 sampling events per plot and 80 actual samples for analysis after bulking

(Table 1). A plot is a GPS-referenced point, also known as a sampling point, where monoliths, cores, pitfall traps and transects are used simultaneously to obtain specimens of soil biota. Typically a plot is positioned randomly, or from a grid system, within a specified land use or land cover, usually a cultivated field or a natural/semi-natural reference habitat. Larger fields could contain more than one plot. At landscape level, separated examples of a land use of interest could be sampled with one or more plots each, depending on the scheme of replication employed. With a recommended replication of three plots per land use, the final figures to be factored into a gradsect (a sequence of land uses aligned to a single environmental variable, generally land use intensification or agricultural intensification; see Gillison & Brewer, 1985) would be 870 sampling events and 240 samples (Table 1). Further bulking can take place during extraction procedures. The scheme gives high point-scale resolution for some groups, especially termites, earthworms and nematodes, all of which are taxonomically tractable, but lower resolution for microsymbionts, not so much because of inadequate sampling but because of taxonomic uncertainties and the requirement for bioassays during the identification procedure. The resolution achieved for macrofauna can be shown by construction of species accumulation curves, and is generally good in longer transects, and by the relative ease of assessing the presence or absence of the main functional groups. Data on abundance and biomass are less reliable because the number and alignment of monoliths does not adequately accommodate the patchy distributions of many macrofauna, or because bulking removes the opportunity to determine variance. Overall, the Swift & Bignell (2001) scheme has high pseudoreplication, and tends towards autocorrelation (Moreira et al., 2008). Where identification to species is not feasible, functional group or higher taxonomic level can suffice (Swift & Bignell, 2001). However, for some groups (for example, macroarthropods such as termites and myriapods) global species richness is relatively low and some individual species have a high impact on soil processes (Lavelle et al., 1997). Therefore identification to species level is desirable. With nematodes, identification to genus level is necessary for deriving the Maturity Index (Bongers, 1990), a parameter which links assemblage structure to environmental disturbance; however with many other

groups of animals and perhaps with microorganisms other than host-specific symbionts and pathogens, functional or higher taxonomic group is as informative as a species list.

Figure 2 A shows a new sampling scheme put forward by CSM-BGBD, a successor project to ASB (Moreira et al., 2008). The minimum scheme illustrated retains the main elements specified by Swift & Bignell (2001) – monolith, core, transect and pitfall trap –, but the overall extent of sampling at each point is reduced and the cores are located on concentric circles around

the single monolith, which is the centre point for the whole sampling array. In other modifications, the number of pitfall traps is reduced to three, coring for mesofauna (12 cores bulked to four or three samples or one sample) is separated from coring for nematodes, microsymbionts, and fungi (also 12 cores, but bulked to a single sample), the length of the termite transect is reduced to 20 m and four soil samples are taken for physics and chemistry, but away from the monolith. The main addition (compared with ASB) is three quadrats for sampling litter to be extracted in Winkler bags, the



**Figure 1.** Transect layout and sampling scheme of Swift & Bignell (2001).

quadrats aligned with the termite transect and pitfall traps. This amounts to 51 sampling events per plot and 22 actual samples for analysis after selective bulking. With a recommended replication of 20 plots per land use, the final figures would be 1,020 sampling events and 440 samples (Table 1). Again, further bulking can occur during extraction. Additional sampling can take place outside the general scheme: for fruit flies (McPhail traps in trees, and from fruit), other flying insects (Malaise traps), termites (casual sampling) and earthworms (handsorting in larger monoliths of 50x50 cm, or application of formol in 1x1 m area to collect larger individuals).

The reduced sampling effort per plot is controversial, largely because the loss of point-scale resolution is large for macrofauna, so an alternative scheme making use of additional monoliths (some larger), more pitfall traps and an extension of the 20 m transect to 50 m has also been used (Figure 2 B). This would take the total number of sampling events to 68 and the number of actual samples up to 59 (1,360 sampling events and 1,180 samples per land use). Separate transects for termites, endogeic ants and Winkler material (Figure 2 A) may not be absolutely necessary, as a procedure for sampling termites, ants, earthworms and beetles simultaneously from a single transect has been described by Jones (2003). In general, the more intensive schemes aimed at the whole biota from

macrofauna to bacteria (TSBF; Swift and Bignell; extended CSM-BGBD) require two complete days of field work per plot, with the digging and sorting of monoliths, and the long transect for termites (and other macrofauna in recent developments of the method, see Jones, 2003) being the time-consuming elements. Increasing the number of field assistants is not usually helpful because of confusion and the danger of trampling damage.

### Selection of sampling points (plots)

Modern sampling campaigns designed to test the hypotheses (stated above) concerning biodiversity and agricultural production at tropical forest margins will normally be structured hierarchically. The ultimate aim is to determine which facets of land use intensification have the strongest influence on below-ground biodiversity and can be used for prediction and future management, and to separate these factors from those which are purely climatic, geographical or edaphic in nature. A benchmark area is first selected (usually in one host country); this should i) represent a recognized biogeographical region, ii) have indigenous biodiversity richness of global interest and iii) have a gradient of significant land uses from pristine forest to intensive agriculture. The benchmark is then sampled with one or more windows (Figure 3), a window consisting of a grid of 2–5 km<sup>2</sup> with about 100 GPS-referenced sampling points defined by intersections, or a set of

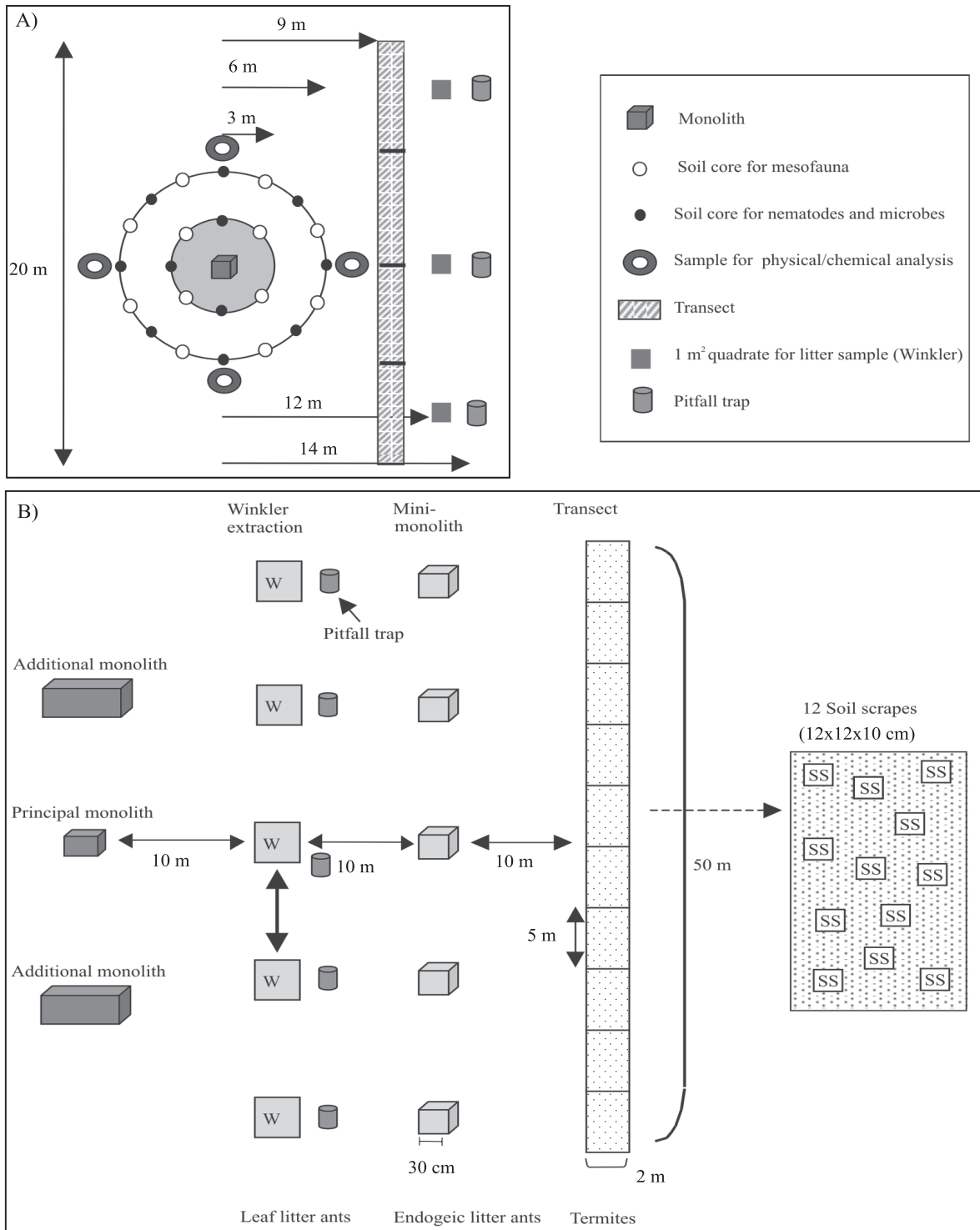
**Table 1.** A summary of the sampling options represented by the three schemes presented.

Sampling parameter	Sampling scheme			
	“TSBF” transect (Anderson & Ingram, 1993)	ASB (Swift & Bignell, 2001)	Basic CSM-BGBD (Moreira et al., 2008)	Extended CSM-BGBD (Moreira et al., 2008)
Methods deployed (sampling events per plot in bracket)	Monoliths (5–10), cores (no. not specified)	Monoliths (10–16), cores (230), pitfalls (20), transect sections (20)	Monoliths (1), cores (28), pitfalls (3), transect sections (4), quadrats (3)	Monoliths (8–9), cores (40), pitfalls (5), transect sections (10), quadrats (5)
No. of biotic groups addressed (groups in bracket)	4 (macrofauna, nematodes, mycorrhiza, rhizobia)	4 (macrofauna, nematodes, mycorrhiza, rhizobia)	8 (additions are mesofauna, saprotrophic fungi, pathogenic fungi, fruit flies)	8 (additions are mesofauna, saprotrophic fungi, pathogenic fungi, fruit flies)
Point-scale arrangement	Linear array	Linear array	Concentric circles + linear array	Concentric circles + linear array
Selection of plots	Not specified	Subjective	From GPS window grid	From GPS window grid
Plots per land use recommended	Not specified	3	20	20
Sampling events per plot (per land use in bracket)	Not specified	290 (870)	51 (1,020)	68 (1,360)
Samples for analysis per plot (per land use in bracket)	Not specified	80 (240)	22 (440)	59 (1,180)
Time required per plot, assuming 6–10 staff available <sup>(1)</sup>	One day or two days, depending on the number of monoliths	Two days	One day	Two days

<sup>(1)</sup>Includes on-site sorting time for monoliths.

separated smaller grids with the same total of sampling points. Each window aims to include up to 6–7 locally significant land uses and to sample each with (ideally)

about 20 points (plots) per land use. A specific benefit of such grid-based schemes is that sampling from a given land use (providing the land use is well defined)



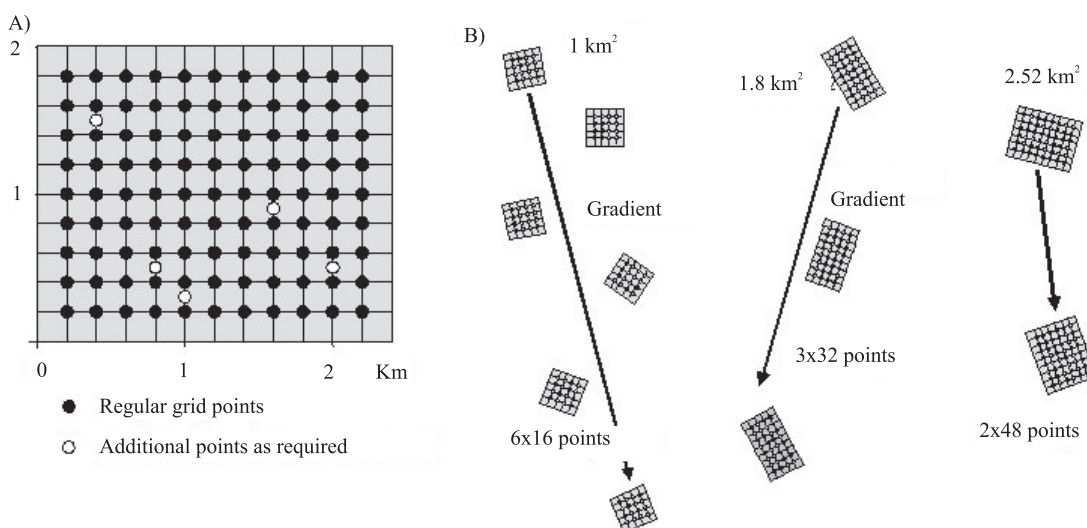
**Figure 2.** Minimum point sampling scheme for all biota adopted by CSM-BGBD (from Huising et al., 2008, with permission of Earthscan) (A). Alternative scheme for sampling macrofauna, making use of a 50 m transect (extended CSM-BGBD; from Huising et al., 2008, with permission of Earthscan) (B). Soil scrapes (SS) sample termites.

can be distributed over a larger area making use of replicate plots that are not part of the same field or other habitat, or in contiguous habitats (Huisling et al., 2008). Points can be added or removed to equalize sample sizes between land uses, or to eliminate points that fall on non-targeted land uses or on transitional zones (unless the latter are of specific interest). Both these measures assist the derivation of valid population means and the estimation of variance for population parameters, an advance from the ASB schemes, which tended to use a smaller number of plots, each of which was more heavily sampled. Windows can also be arranged along a gradient, such as altitude, distance from the forest margin, or land use intensity. This converts the sampling exercise into a gradsect and is one example of stratification, i.e. a modification of sampling procedures which reduces the scope of a study but sharpens its focus. It is not usually practical to sample without a priori hypotheses, i.e. to sample a benchmark entirely at random and then look post hoc for the variables which best explain the patterns of soil biodiversity observed. The separation of prospective sampling points on a grid system is not specified in the CSM-BGBD protocol, except that the total number of points used in each benchmark investigated should be about the same. This is deliberate, to allow different landscape configurations to be accommodated, for

example: a system of small fields might require points to be closer together to sample all the significant land uses; however a useful rule of thumb is that a separation of at least 200 m is desirable, to reduce autocorrelation, and 100 m is the minimum acceptable. Patchiness of below-ground biodiversity cannot be determined on a scale less than the separation of the sampling points, but within-field variations are not priorities for research in international aid projects aimed at creating food security (Huisling et al., 2008).

### Grids and transects compared

Theoretical sampling schemes for natural biotas are described in many textbooks (Cochran, 1977; Ford, 2000; Southwood & Henderson, 2000; Gregoire & Valentine, 2007). The practical priorities are a rapid low-cost protocol, co-location at point level for sampling all biotic groups and measuring abiotic variables, independent repetition of treatments in order to produce consistent patterns, low variance within treatments to improve detection of differences between treatments and sufficient sampling points per treatment to permit a statistical test of hypothesis. Meeting these requirements is difficult, because of the paucity of previous data from which to judge the likely magnitude of variance, the taxonomic intractability of many of the biotic groups addressed and, not least, the nature of international development projects themselves, where



**Figure 3.** Examples of configurations of sampling windows. A full grid with additional sampling points added (open circles) (A); separation of a full grid into differing schemes of smaller windows, which can be aligned with landscape gradients (B). The total number of sampling points is the same in each case. From Huisling et al. (2008), with permission of Earthscan.

objectives can be ambitious and sometimes not matched by the resources allocated for actual field work or the expertise available for the identification of specimens. It is rather easier to identify what is not wanted in a sampling scheme: pseudoreplication (in effect multiple sampling from the same field), autocorrelation (spatial correlation), unwieldy datasets containing a large number of zeros (monoliths, cores, traps, transect sections or other subsamples not containing the targeted groups), ambiguous definitions of land uses (and confusion with land covers), and excessive within-treatment variability. Fortunately, some basic tools are available to judge the adequacy of sampling, of which the most useful is the species accumulation curve (Colwell, 1997). Another useful tool is stratification: if some sources of variability can be identified in advance, they can be used to define strata and removed from the analysis, for example, altitude zones. By this definition, one stratum therefore consists of a set of points, embracing several land uses, which are geographically close. Finally, sampling cannot be expected to generate a single numerical index of below-ground biodiversity, nor will it yield every type of organism in the landscape.

Systematic sampling is usually defined as sampling to some predetermined scheme. Grids and transects are the main examples. In the projects mentioned above, sampling is designed to test the hypothesis that below-ground

biodiversity differs between land uses (e.g. forest x crop field), rather than between different parts of the same land use (e.g. field centers x field margins). This requires that the sampling should be systematic (in the above sense), because the land uses selected are not independent of one another, although small grids and transects can be randomly positioned (randomly oriented in the case of transects) within each land use, and replicated among different plots within the same land use. Descriptive statistics based on a random design can then be used for the data from each land use, but comparisons between land uses are more problematical.

The ASB to CSM-BGBD transition reflects a debate about the advantages and disadvantages of grids and transects, which has not been entirely conclusive. Table 2 summarizes the main features of the two sampling systems. It is predicated on the assumption that the ASB methods are intensive per point sampled (and therefore have high resolution for actual biodiversity), whereas CSM-BGBD methods are less intensive per point but better replicated over the same land uses in any given benchmark. The main issues are as follows:

- i. Representation of land uses. Grid based schemes spread the sampling more thoroughly through the benchmark.
- ii. Autocorrelation. It is probable, a priori, that autocorrelation between sampling points will affect

**Table 2.** Relative merits of grids and transects. In practice, different styles of sampling can be combined, e.g. stratified random, stratified non-random (systematic), nested systematic, random systematic, but the main issue in designing sampling campaigns is between transects and grids.

Sampling parameter	Transect-based (few plots, higher effort per plot)	Grid-based (many plots, lower effort per plot)
Ease of positioning samples, access to sites and field logistics	Higher	Lower
Field time	Shorter	Longer
Representation of the land use distribution	Less good, normally more subjective; concentrates sampling, but rare land uses can be chosen	Better, normally derived from remote sensing; spreads sampling, but rare land uses may be missed
Autocorrelation	High	Low
Stratification	Possible	Possible
Sensitivity to aggregated species	Low	High (better than random sampling)
Precision of biodiversity sampling	Higher	Lower
Replication	Pseudoreplication	Genuine replication
Redundant points	Few, if any	Some
Estimations of abundance and biomass	Not possible (or high variance)	Possible (or low variance)
Adaptability to landscape land use distributions	Low: a transect cannot usually sample two land uses at once	High: sampling points can be added or subtracted
Adaptability to small plots	Possible	Extra points can be added
Estimations of functional group representation	Good	Possible
Co-location of samples	Implicit	Possible
Samples with no biota (zeros)	Fewer	Greater
Supplementation by casual sampling	Unnecessary	Desirable
Point-scale disturbance and risk of sample contamination	High	Low



the conclusions drawn from any ecological study (Legendre & Legendre, 1998), but the extent to which this is a serious impediment in soil biodiversity studies is unclear. However, referees of submitted scientific manuscripts can be harsh in judging the issue. Stationarity theory argues that mean and variance for parameters such as species richness, functional group diversity, abundance and biomass will not change overall within a window, such that the level of correlation between point samples is always the same, although individual points will vary in their similarity to each other over time (Huisling et al., 2008).

iii. Precision of biodiversity sampling. This is higher for longer transects and transect-aligned monoliths, because it takes better account of the known patchiness of soil animal distribution.

iv. Replication and estimates of abundance and biomass. Transects with few sampling points, for example 5–8 monoliths, typically generate abundance and biomass means with large variance (Bignell et al., 2000), which then restricts the demonstration of significant differences in below-ground biodiversity to treatment extremes only (for example pristine forest vs *Imperata* grassland), an effect which can in any case be easily seen by inspection without the need for extensive sampling. It is currently unclear whether an increase in the number of sampling points improves resolution, in the sense of reducing the large variances. The greater number of sampling points inherent in the grid-based schemes should improve statistical resolution between treatments, but firm experimental evidence of this is lacking. Huisling et al. (2008) recommend 100–120 sampling points per benchmark, with a typical benchmark containing up to six land uses of interest. However, some information can be extracted from the relatively low-replication data generated in transect-based studies by the use of multivariate analysis (Eggleton et al., 2002) or by the relative balances between different functional groups (Jones et al., 2003). The use of transects permits some quantitative (or semi-quantitative) data to be gathered, but does not exclude qualitative analysis, as is (for example) traditional with ants.

## Conclusions

1. Modern multi-taxon sampling schemes for below-ground biotas employ combinations of monoliths, cores, pitfall traps and transects.

2. This mixture provides for co-location of sampling for macrofauna, mesofauna, microfauna, microsymbionts and other microbiota, and a higher resolution of below-ground biodiversity than can be achieved by point-scale or transect-based schemes alone.

3. Where logistics permit, selection of sampling points should be grid-based, on the assumption that this provides the best representation and replications of land uses, and the most robust statistical descriptions of data, i.e. lower variances and higher resolution between treatments.

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