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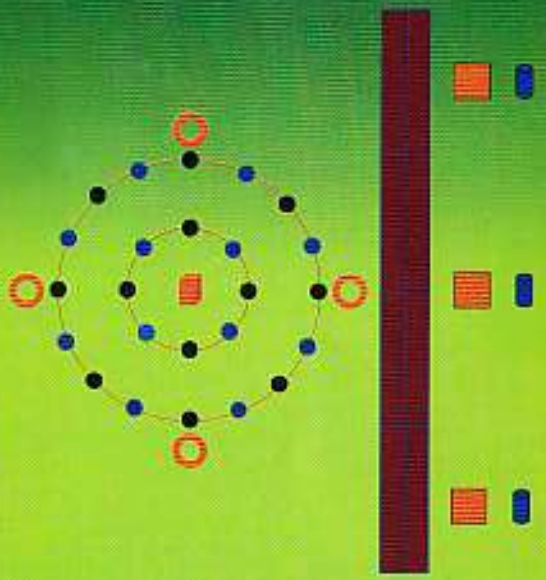
This book contains methods for assessing below-ground biodiversity in the tropics. Field sampling (rapid assessment) and identification (up to the lowest possible taxa and/or functional group) of selected below-ground biota are presented, i.e. macrofauna (*ants, beetles, earthworms,* and *termites*), nematodes, mesofauna (*collembola*), and microbes (*legume nodulating bacteria, arbuscular mycorrhizal fungi, saprophytic/ decomposing fungi,* and *plant pathogenic fungi*).

Examples of data analyses and illustration are also given. Measurement of soil properties is added to seek the relationships between the diversity or abundance of below-ground biota and its soil environment. The book will be suitable for researchers and students interested in studying and/or conserving biodiversity.

METHODS FOR ASSESSMENT OF BELOW-GROUND BIODIVERSITY IN INDONESIA



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F.X. Susilo
Agus Karyanto

UNIVERSITAS LAMPUNG
Bandar Lampung

This publication is part of the outcomes of the international project "Conservation and Sustainable Management of Below-Ground Biodiversity (CSM-BGBD)" executed in seven tropical countries – Brazil, Cote d'Ivoire, India, Indonesia, Kenya, Mexico, and Uganda. The project is coordinated by the Tropical Soil Biology and Fertility Institute of CIAT (TSBF-CIAT) with co-financing from the Global Environment Facility (GEF), and an implementation support from the United Nations Environment Programme (UNEP).

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FOREWORD

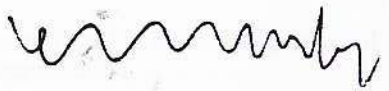
I am very pleased to introduce this book of Methods for Assessment of Below-Ground Biodiversity in Indonesia. This publication offers (more or less) standardized methods for evaluating below-ground biodiversity (BGBD) in Indonesia, covering biodiversity measurement, the development of sampling protocols, and field sampling procedures for BGBD inventory. The book describes sampling and specimen identification of selected below-ground biota, i.e. earthworms, termites, ants, beetles, nematodes, collembola, and microbes (legume nodulating bacteria, arbuscular mycorrhizal fungi, saprophytic-degrading fungi, and plant pathogenic fungi). Examples of data analyses are also presented. I am sure it will prove to be interesting and useful reading for BGBD researchers.

In this occasion, I would like to thank GEF-UNEP, TSBF-CIAT and CSM-BGBD Project for supporting this BGBD research in Indonesia. I also thank F.X. Susilo and Agus Karyanto, our faculty members, for their editorial assistance to accomplish the publication of this book.

I believe that this book is beneficial not only as reference for BGBD researchers but also for students who carry out research on the above topics.

Bandar Lampung, June 2005

Rector of University of Lampung,



Prof. Dr. Muhajir Utomo

PREFACE

The diversity of below-ground organisms, i.e. bacteria, fungi, protozoa and invertebrate animals, is enormous. Yet little attention has been given to the studying of the below-ground biodiversity (BGBD) possibly due to its unseen nature and difficulty in assessing them. So far, especially in the tropics, the focus of studying biodiversity is limited on above-ground interaction especially aimed at sustaining food production.

Very limited data of below-ground organisms are available in the tropical region where its biodiversity is believed to be the highest on earth. The scarcity of the data is partially due to the absence of agreement on standardized methods for the study of BGBD. Despite its importance to ecosystem function, in many cases the soil community has been almost totally ignored in considerations of biodiversity conservation and management even at the inventory level. The information about the current status, the value perceived by the various sectors of society, and the factors which drive change in one direction or another are important for sustainable and profitable management of agricultural biodiversity, including BGBD.

This book tries to supply methods for assessing BGBD that have been tested in seven tropical countries (Brazil, Mexico, Kenya, Uganda, Ivory Coast, India, and Indonesia). With such a "standardized methods", the inventory results of BGBD should be comparable among regions, furthermore, the generalization of the trend for BGBD loss, as imposed by land use change from forest into agricultural land, would be possible.

The sampling method in this book is originally designed for assessing various below-ground organisms simultaneously, i.e. for studying biodiversity in any given area. Nevertheless, anyone who is interested in studying just one or two particular below-ground biota is hoped to find this book useful.

We hope that this book will be useful for anyone who deals with biodiversity study and in turn could contribute to the efforts of raising awareness for conserving BGBD in Indonesia.

We also sincerely acknowledge that this book is far from complete and any suggestion and comment will be highly appreciated.

Bandar Lampung, June 2005

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1 Introduction

1.1 What is Biodiversity?

Biodiversity or biological diversity according to The Convention of Biological Diversity (CBD) means *the variability among living organisms from all sources including,* inter alia, *terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.* Perhaps the simplest explanation of biodiversity is the variety of life in all its manifestation.

The below-ground biodiversity (BGBD), which dealing with all biotic components and their interaction with nonliving things in the soil, is an integral part of biodiversity. This can be perceived that above-ground activities are closely related to below-ground entities, i.e. soil supports almost all living things on earth. However, there is a growing concern that the variety and complexity of life within the soil need to be quantified with valid methods in order to be able to make comparative analyses of changes in BGBD across time and space.

1.2 Measuring Biodiversity

Due to the variety of elements that make up BGBD, there are many ways of measuring it. In theory, any element that can be recognized as a discrete unit (e.g. genes, individuals, species, and families) can be used to measure diversity. Species richness can be viewed as the most straightforward measure of diversity (Gaston, 1996), and can be simply defined as the number of species in an area. However, this measure of diversity is considered to be of rather limited value because it does not take into account any differences among species. In ecological terms, the number of species and the number of individuals in each species (known as abundance) are regarded as the two most meaningful components of species diversity. In mathematical terms, diversity should give a measure of how the individuals present in a sample are distributed among the species in

that sample. In an effort to express species diversity as a single numerical value, scientists have created many diversity indices that use both species richness and abundance. However, all these indices are subject to some form of bias that can make their use inappropriate under certain circumstances. For instance, many are strongly influenced by sample size, while others are skewed in favor of rare species or dominant species. The accurate biological interpretation of a numerical value derived from any index can be difficult, so it is often easier and more straightforward to present the basic information of species richness and abundance (Jones, 2003).

1.3 The Need for Standardized Sampling Protocol

The sampling protocol has to be designed that can get meaningful data on local biodiversity with minimum effort allowing for the frequent constraints imposed by inadequate resources (time, money, and taxonomic expertise). Both technical and logistical consideration must be made especially for monitoring and conservation purposes where there is a necessity to gather information from a relatively large number of sites. Hence, the more "rapid" a sampling method is, the more sites can be assessed in the available time (see Jones, 2003). However, any rapid sampling protocol will involve a compromise between trying to minimize the size of the samples in order to reduce the time spent collecting and sorting, and the need for samples that are large enough to give a result that is representative of the local sites.

Another important consideration of any sampling program is to ensure that standardized collecting and processing methods are adopted at all times, and by all personnel involved. Thus, the use of standardized sampling methods allows direct and accurate comparisons to be made among sites.

Given the variety of below-ground organisms ranging from microscopic organisms to visible macrofauna, it is impossible to have a sampling method appropriate for all soil organisms. Even among macrofauna, e.g. termites, ants, beetles, and earthworms, there is no single sampling method suitable for all groups of those soil invertebrates (Jones, 2003). Thus, when sampling a wide range of soil biota for conservation or sustainable management purposes, there is a need to comply with the standard sampling protocol.

1.4 The Development of BGBD Sampling Protocol

Although methods for assessment of BGBD have been described in TSBF – A handbook of methods by Anderson and Ingram (1993) and ASB lecture note 6B by Swift and Bignell (2001) some of them need updating or more details. The sampling method for studying below-ground biodiversity in the Conservation and Sustainable Management of Below-ground Biodiversity (CSM-BGBD) Project is actually derived from the "TSBF method". The TSBF method exhibits a basic field transects of 40 x 4 m for macrofauna inventory as recommended by Anderson and Ingram (1993) and this method was widely used during the Alternatives to Slash and Burn Program (ASB) Phase II (1977).

Within the TSBF transect usually lies 5"10 monoliths. In regard to monoliths' placement as well as their number, some suggestions have been made; monoliths can be placed within transect, in a grid system, or in randomized order. A grid system is regarded as the main alternative to transect. The lay out of monoliths in a grid is considered more satisfactory from the statistical point of view than that of within transect. In most cases, the data from 5"10 monoliths within transect can only be regarded as a single data point, whereas in a grid system the data from 5"10 monoliths are true replicates (Swift and Bignell, 2001). Other option to place monoliths is in randomized order within the plot/site. The later arrangement is intended to accommodate the view that any generation of quantitative data (abundance, biomass etc) should be strictly randomized. The choice of the method, however, should depend on local circumstances and/or the type of data required.

Other than monoliths, soil cores were often used to collect macrofauna. However, there has been a consensus that soil macrofauna is best sampled by few monoliths rather than by many cores. It is believed that the probability for not finding any animals in the monoliths is lower than that in the cores. This is mainly attributed to the dimension of the monolith of 25 x 25 x 30 (depth) cm which is larger than that of the cores that usually between 2 cm to 10 cm. Furthermore, data derived from cores are typically more difficult to handle because of the very large number of zeros (see Bignell and Eggleton, 2000). Therefore, monoliths are preferred.

In its development, the transect-based sampling is better supplemented with some other methods to assess aspect of biodiversity which the TSBF transect may not cover. The longer version of transect up to 100 m was then proposed and it could be placed parallel to the TSBF transect at about 10"20 m separation.

The second transect is suited for sampling termites as described in Swift and Bignell (2001). The 100 m transect is divided into 20 sections of 5 m, each of which is sampled for one person-hour. The data generated are considered semi-quantitative (Jones and Eggleton, 2000).

In addition to transect, other sampling methods such as pit-fall traps and Winkler are introduced. A set of pit-fall traps is intended to sample highly mobile invertebrates. Pitfalls of about 10"15 cm diameter are arranged in parallel to the transect line. The depth of the traps is not critical, but should be at least 5 cm. Unbaited pitfall traps are simpler to prepare and set than those of baited pitfalls, and hence unbaited pitfalls are considered mandatory while baited pitfalls are optional for sampling scheme.

The use of standard Winkler bags (Agosti *et al.*, 2000) is intended to extract ants and beetles from leaf and small woody litter, sampled on and off transect. The Winkler is probably the best of all the add-ons, as it meets the TSBF objectives of being both quantitative and replicated (Swift and Bignell, 2001).

Thus, methods for macrofauna sampling should include monoliths, pitfalls, semi-quantitative transect,[1] and Winkler. Monoliths, cores (for nematodes and microsymbionts), and soil physical and chemical measurements should be co-located in an area of 200 m^2 around the sampling point. ***The area can be the traditional TSBF/ASB 40 x 5 m transect or a circle of radius 8 m^2.***

The sampling protocol with the monolith as a center point in a circle of radius 8 m is then chosen[3] as a basic design considering, among others, that not all land uses having such a large area and the necessity to have at least five sampling points per land use. The circular design seems more appropriate to study biodiversity in various sizes and types of land uses, and its layout is shown in Figure 1.

[1] Note: transect and monoliths are different kinds of sampling and the data from each should be kept separate

[2] Global Workshop Report 2003 on Conservation and Sustainable Management of Below-Ground Biodiversity (CSM-BGBD), February 2003, Sumberjaya, Lampung, Indonesia.

[3] Annual Meeting Report 2004 on Conservation and Sustainable Management of Below-Ground Biodiversity (CSM-BGBD), February 2004, Embu, Kenya.

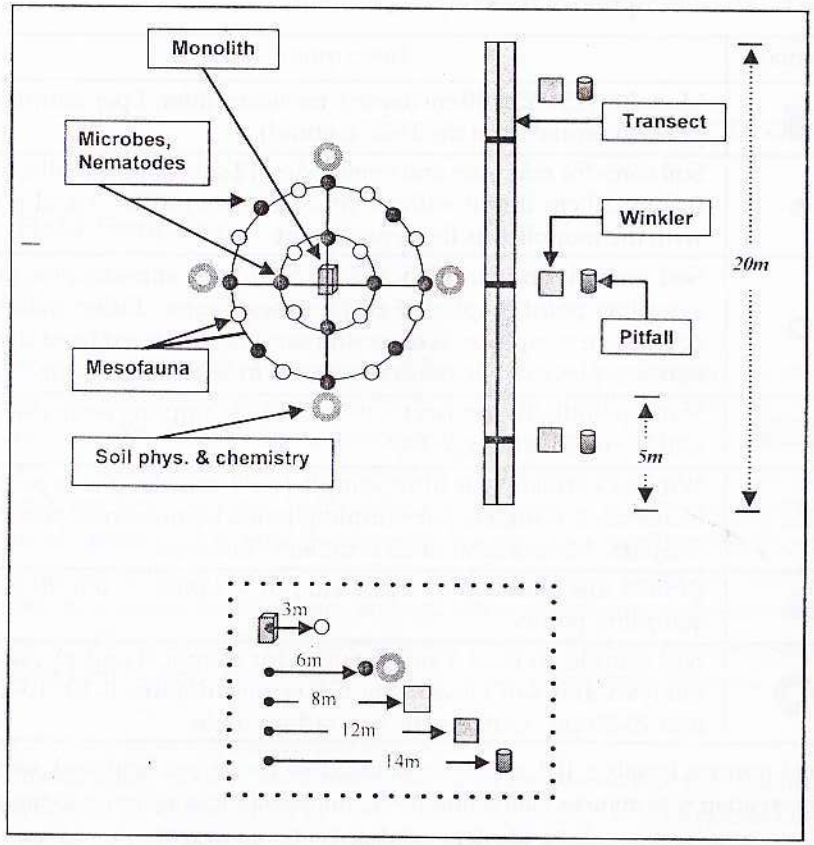

Figure 1. Layout of BGBD sample point.

Table 1. The description of the sampling symbols

Symbol	Description
	Monolith (25 x 25 x 30 cm depth), including litter, 1 per sampling point (adapted from the TSBF method).
	Soil cores for microbes and nematodes, 12 sub-samples collected from 0-20 cm depth without litter. Circles radius: 3 and 6 m with the monolith as the center point.
	Soil cores with jitter (3.5 X 3.5X 5 cm), 12 sub-samples per sampling point (depth 0-5 cm) for mesofauna. Either make 1 composite sample or 3 composite samples collected from the 4 sub-samples of each quadrant of the circle of 3 m and 6 m.
	Semi-quantitative transect, 2 x 20 m, 1 per sampling point (Swift and Bignell, 2001; page 14)*.
	Winkler extractor for litter sample (1 x 1 m), along side of the transects, 3 Winklers per sampling point, equidistance about 6 m apart. Make a total of 15 Winklers / land use.
	Pitfalls are placed 1 m outer side of Winkler, 3 pitfalls per sampling points
	Soil sample, at least 4 sub samples for chemical and physical analysis and soil classification (coring), depths: 0-10; 10-20; and 20-30 cm. Outside the 6 m radius circle.

*) total transect length = 100 x 2 m /land use (five points per land use); section observation = 30 minutes (soil profiles) + 15 minutes (micro niches) = 45 minutes /2 persons/section, please see detailed description on page 8.

Table 2. The below-ground biota groups to collect from each sampling method

Sampling method	Below-ground biota groups						
	Ants	Ter-mites	Beetles	Nema-todes	Earth-worms	Meso-fauna	Microbes
Monolith	X	X			X		
Soil core*				X			X
Soil core*						X	
Transect		X					
Winkler	X					X	
Pit-fall	X					X	

* see Figure 1 and Table 1 for the description of each soil-core sampling.

2
Macrofauna

2.1 Field Sampling

Monolith

A monolith, as defined for the purpose of below-ground biodiversity inventory, is a block of soil sample of dimensions 25 x 25 x 30 (depth) cm taken from the inventory site (Anderson and Ingram, 1993; Swift and Bignell, 2001). One monolith is taken per sample point and at least five sample points are taken randomly per land use type. In the field, the monolith is placed at the center of the sample point and is thus used as the reference for positions to do other sampling methods (transect, Winkler, pitfall, and soil cores for sampling microbes, nematodes & collembola, and soil physical & chemical characteristics) (Figure 1). The monolith procedure is aimed at sampling soil macrofauna including earthworms, ants, beetles, termites, and other invertebrates.

The procedure of doing a monolith is as follows.

1. A metal frame of size 25 x 25 cm is placed on the soil surface marked for the monolith, i.e. at the center of a selected sample point and as referenced by the GPS. Litter thickness (in cm) is measured at five locations inside the frame. Litters inside the frame are placed into a plastic tray, and any spotted invertebrates are collected. Earthworms are picked up using a forceps and put into a vial filled with 15"20 ml of 4% formalin, whereas other specimens are picked up using a small brush or forceps and put into a vial filled with 15"20 ml of 70% ethanol.
2. After litters are removed from the soil surface, soil temperature is measured at 0"10cm depth by inserting three thermometers into the monolith surface at three different positions diagonally inside the frame; letting them steady for 15 minutes for reading.
3. The monolith is isolated by digging a 20-cm wide x 30-cm deep trench around the 25 x 25 cm metal frame. The exposed monolith block is then divided into three layers, i.e. 0"10 cm, 10"20 cm, and 20"30 cm. Soil

from each layer is placed in a plastic tray and hand-sorted *in situ* to collect earthworms and other soil invertebrates.

4. Soil temperatures are also measured at 10"20 cm and 20"30 cm depths.
5. Upon arrival in the base-camp or laboratory, earthworms are washed thoroughly with clean water, and stored in vial filled with 15"20 ml of 70% ethanol. During washing, the number of earthworms is tallied and hand-sorted for intact- versus broken- individuals. All samples are then ready for taxa identification and biomass estimation.

Transect

This is aimed at sampling termites. A transect is basically a rectangle plot of dimensions 2 x 20 m inside of which termites are collected. Five transects are taken per land use type which give a total size transect of 2 x 100 m per land use type (BGBD Global Workshop II Report, 2003). The transect is laid perpendicular to and at 8"10 m distance from the monolith center (Figure 1), i.e. along an area most probable for finding termites (for instance, along the upper slope in case of sloppy sampling area). In each transect, four sections of dimensions 2 x 5 m are assigned, i.e. section 1 (S1), S2, S3 and S4. Within each section, a right- and a left- sub-sections of 1 x 5 m are also assigned. For simplicity, the right sub-section is the one nearest the monolith.

Termites are collected inside each of the 2 x 5 m section in 1.5 person-hours (Susilo and Aini, 2005). One person-hour (2 persons each for 30 minutes search per section) is spent to collect termites from the soil (scraping) and an additional half-person hour (2 persons each for 15 minutes per section) from various micro niches. A trowel is used to take 12 soil scrapes (each of dimensions 12 x 12 x 10 depth cm) per section (or 6 soil scrapes per person per section). The scraped soil is placed into a plastic tray and sorted for termite *in situ* using a small brush or sharp pointed forceps. Termite specimens (soldier caste as priority) are collected into glass vials containing 70% alcohol solution for preservation and identification. Specimens encountered in the same soil scrape are collected in the same vial. A pencil-written (or permanent drawing pen) paper is used for label and placed inside the vial. The information in the label includes the date, transect section, origin of specimen (i.e. soil scrape or specific micro niche), code of sample point and land use, and the name of the collector. The allocated 1.5

person-hour time is used for the whole scraping process including scraping, putting the scraped soil into the tray, sorting, putting the termite into vial, and labeling. Micro niche collection within the transect section is conducted following the scraping collection. The micro niche collection did not involve soil diggings; instead, encountered termites are collected directly using small brush or a forceps from various micro niches including litter, galleries, tree root buttresses, dead wood, mounds, stumps, or arboreal nests (at a maximum height of 2 m). The dead wood, mound or stump are opened up or broken using a machete and any spotted termite is collected. Specimens found from the same micro niche are placed in the same vial. Prior to identification process, all termite specimens in the vials are subjected to cleaning and re-labeling.

Winkler

The Winkler is used primarily to sample soil and litter ants, beetles (Agosti *et al.*, 2000; Chung *et al.*, 2000), and other invertebrates. The Winkler apparatus consists of five components (Jones *et al.*, 2003), i.e. a Winkler bag with internal wire frames, mesh bags of 2 mm mesh, a sieve cloth of 1 cm^2 mesh, a debris bag, and a collecting jar. The internal wire frame consists of two wire rectangles (30 x 25 cm) that are set 50 cm apart. Below the lower wire frame, the Winkler bag is funneled into the collecting jar.

In each sample point three quadrates of 1-m^2 surface litter are collected (Figure 1). The quadrates are located 6 m apart from each other, lined parallel to, and at 2 m from the 2 x 20 m transect as such that the distance of the Winkler samples to the monolith is 12 m. In each land use type, five sample points (5 x 3 of 1-m^2 surface litter) are taken.

Litter samples are collected on bright mornings. Catches may not be representative if collection is done on rainy day or wet condition (due to rain on the previous night) (Chung and Jones, 2003). Small ants or beetles could be trapped in the water layer on the litter. Wet soil and litter may lump together and get trapped (and thus trap the ants and beetles) in the sieve, resulting in low effectiveness of the sampling.

The litter and surface soil are scraped up to a depth of anywhere between 1 cm to 3 cm with gloved-hands. Scraping is done quickly from the edges of the quadrate towards the center to prevent the insects from escaping. The litter sample is then poured into the sieve cloth and sieved vigorously to exclude larger elements (e.g. twigs, large litters, stones) and let the smallersized litter to pass.

Decayed twigs can be broken up by hand and put into the sieve. The sieving will usually be done by two persons for about three to five minutes per quadrate. The resulting litter sample (i.e. sieved materials with ants and beetles in it) is collected into the debris bag (a pillow case is very suitable) for transport and later extraction.

At the base camp or laboratory, the litter sample (weighing between 2"3 kg) in the debris bag is transferred to the mesh bags and suspended in the Winkler bags. Mesh bags should not be overfilled. While loading the samples into the mesh bags, a tray is placed below to catch any falling debris. Each Winkler bag (the outer bag) can contain a maximum of four mesh bags. Only sieved material from one quadrate should go in each Winkler bag. If the volume of sieved material fills more than four mesh bags, then a second Winkler bag must be used. There is a need to ensure that mesh bags do not touch each other when hanging the Winkler bag. The Winkler bags are left suspended at room temperature for 72 hours. During that period the litter air-dries up. Ants, beetles, and other invertebrates that trying to escape from drying litter inside the Winkler bag will fall into a collecting jar filled with 75% ethanol solution at the bottom. The invertebrates from the collecting jar are then transferred to vials for labeling, storage and identification. Penciled (or permanent drawing pen) label included the Winkler number, sample point name, land use type, dates, name of collector is placed inside the vial together with the specimens.

Pitfall Trap

This is a kind of un-baited pitfall trap. This trap is to sample ants, beetles, and other below-ground invertebrates including springtails (collembola). Three pitfall traps are set at 2 m from the Winkler samples or 14 m from the monolith center (Figure 1). Pitfall trap, made of a 13-cm diameter plastic bucket, is set in the soil with its opening parallel to the soil surface. The trap is filled with 200 ml of 1% detergent solution. A plastic roof of dimensions 20 x 20 cm is placed 15 cm above the mouth bucket to prevent the rain falls directly into the bucket. The pitfalls are set in the field for 24 hours. Surface dwelling invertebrates that wander around fall randomly into the trap and sink into the detergent solution due to low surface tension. Thereafter, the bucket is emptied and any invertebrates caught along with the detergent solution are transferred into plastic bag and labeled for its location (i.e. sample point name), date, land use type, and the name of the collector.

Upon arrival in the base camp or laboratory, the catches are washed with water to remove the detergent. Larger invertebrates (ant, beetles, and others) are stored in vials filled with 75% ethanol and ready for identification. Meanwhile, collembola should be filtered with collembola filter (100 mesh) before being transferred into vial filled with 96% ethanol and labeled as such.

2.2 Specimen Identification

Earthworms

All earthworm specimens from monolith are identified. The identification is based on their external and internal morphology. External morphological characters to be observed include the number of segments (clitellum segments, segments anterior to clitellum), the type of clitellum and genital pore, the type of prostomium, setae arrangement, and tail form. The internal characters (gizzard type, calciferous gland, prostatic gland, the type of nephridia, intestine) are also observed (on the dissected specimens) (see Figure 2). All specimens are identified up to species level under a dissecting microscope using Sims and Easton (1972), Reynold and Righi (1994), and Jones (2003). The earthworm data (the list of species with corresponding density in each sample point) can also be re-arranged based on to the origins, i.e. native versus exotic groups (Fragoso *et al.*, 1997). Following identification, the specimens are weighed to document their biomass (based on blotted preserved specimens, Swift and Bignell, 2001).

Termites

The identification of termite specimens is based on their external morphology, mainly the morphology of the soldier head and the worker mandibles (Homathevi, 2003a; 2003b). Identification is done under the dissecting microscope using Tho (1992) and Thapa (1981) as the main source of reference. Other references that could be used include Homathevi (2003a; 2003b), Jones *et al.* (2003), and Ahmad and Akhtar (1981). The termites are identified up to the species level. The data are also arranged based on the feeding groups. We note the four feeding groups of Donovan *et al.* (2001) and others (Homathevi *et al.*, 2002 and Eggleton *et al.*, 1997), but simplify them into two groups as attempted by Gathorne-Hardy & Jones (2000) and Gathorne-Hardy *et al.* (2001), i.e. wood feeders and soil feeders. For some species with unknown feeding groups, the feeding group is assigned using gut color as

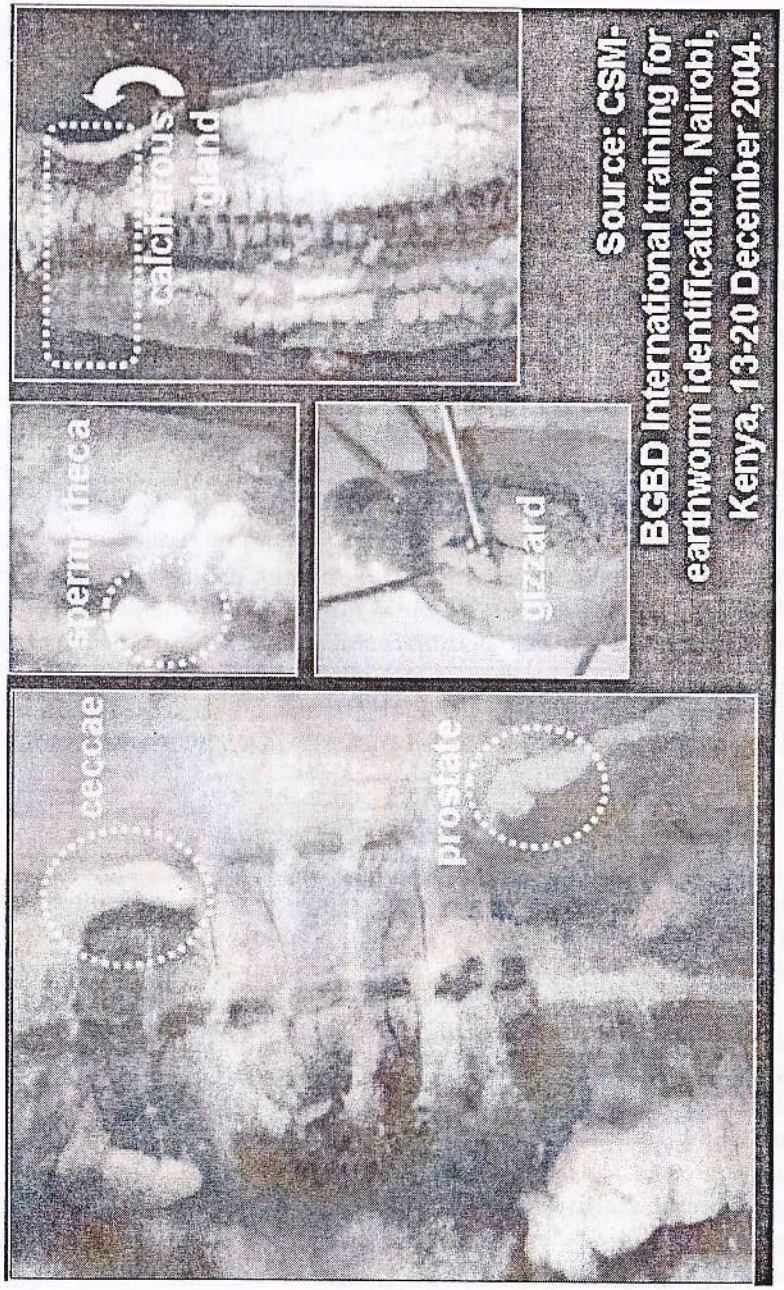

Figure 2. Internal morphology of earthworm as a means of specimen identification.

indicator (dark as soil feeder and light as wood feeder) and taxonomic affinity to species with known feeding group. Termite specimens to be identified include those from the monolith and transect sampling. Following identification, the specimens are weighed to document their biomass (based on blotted preserved specimens, Swift and Bignell, 2001).

Ants

The identification of ant specimens is based on their external morphology of the worker caste. Key morphological characters to be observed include the petiole (number of separate nodes), antenna (segment number, club segments, scape length), mesosoma (spine or tooth, propodeal spine or tooth in side view), eye (size and shape, location in frontal view), and gaster (size of tergum, color). Identification up to genus level was done under a dissecting microscope using Hashimoto (2003) and Bolton (1994). The images of ants of Sumatra in Alpert and Susilo (2005) are consulted for confirmation. The data are also arranged in feeding groups, i.e. predator, forager, and others (Hashimoto, 2003; Brown Jr., 2000). Ant specimens to be identified include those from the monolith, Winkler, and pitfall sampling. Following identification, the specimens are weighed to document their biomass (based on blotted preserved specimens, Swift and Bignell, 2001).

Beetles

The identification of beetle specimens is based on their external morphology of the adults. The morphological characters to be observed include the rostrum (presence), antenna (type, number of segments), mouthparts (position: hypognathous, prognathous), pronotum (coverage over the head, presence of lateral margins), elytra (coverage over abdominal terga, striation, presence of hairs), hind coxae (coverage over the first abdominal sternum), body form (ant-like, elongated, oval). Identification up to family (and some to subfamily) level was done under a dissecting microscope using Chung (2003) and Borror *et al.* (1981). The data are also arranged in feeding groups. Out of 6 feeding groups identified in Chung *et al.* (2000) which followed Hammond (1990), we simplify them into four feeding groups, i.e. herbivores, predators, scavengers, and fungivores. Beetle specimens to be identified include those from the monolith, Winkler, and pitfall sampling. Following identification, the specimens are weighed to document their biomass (based on blotted preserved specimens, Swift and Bignell, 2001).

Other Invertebrates

The other invertebrates referred to in here include those below-ground animals other than earthworms, termites, ants, or beetles that are found from either monolith, transect, or Winkler. Key morphological characters to be observed include the body segments (number), antenna (presence, number), compound eye and ocellus (presence, number, position), mouthparts (type), legs (presence, number, number of tarsal segmens), wings (presence, number), and other appendages (cerci, caudal or lateral filaments). This group is identified up to order or higher levels under a dissecting microscope using Mohamed (1999) and Borror *et al.* (1981). Specimens of the other invertebrates to be identified include those from the monolith, Winkler, and pitfall sampling. Following identification, the specimens are weighed to document their biomass (based on blotted preserved specimens, Swift and Bignell, 2001).

2.3 Data Compilation and Presentation

The inventory results in two types of data, i.e. the biological and ecological data " in Longino (2000) the term "taxonomic data" is used for the "biological data". The biological data include the taxa (i.e. the list of names of species, genus, families/subfamilies, order or class of the below-ground macrofauna), their functional groups, and density (population, biomass) while the ecological data include the time (dates, season) and place of collection (geo-referenced sample point, land use type), and others (including sampling methods). The aim of data compilation and analysis is thus to relate between the biological data and the ecological data so that we can make sense out of it (new scientific information) and can do something about it (technical and applied information). A question can be asked to initiate the data analysis, for instance "What is the relationship between (the effect of) land use types and (on) the diversity and the density of the below-ground macrofauna?"

The biological and ecological data at hand are first compiled as raw data in a spreadsheet. The taxonomic list data are entered in the first two columns of the spreadsheet while the density data per sample point per taxa are entered in the subsequent columns. The sample points are arranged consecutively per land use type. The other data are placed at or as part of the title or note of the spreadsheet. As an example, a table showing the

(hypothetical) raw data of termite numerical density from monolith (Table 3) is presented (this is just an example; another table, albeit more complicated one, can also be set, i.e. where data in each sample point are further allocated per monolith layers – litter, 0-10 cm, 10-20 cm, and 20-30 cm). Table 3 shows the number of each species of termites found in each sample point, i.e. per monolith (pooled at the whole 30 cm depth). Similar tables can be set for termites (number of encounters of each species of termites found in each sample point, i.e. transect), ants (number of each genus of ants found in each sample point, i.e. per monolith, per 3 Winklers, and per 3 pitfalls), beetles (number of each family/subfamily of beetles found in each sample point, i.e. per monolith, per 3 Winklers, and per 3 pitfalls), earthworms (number of each species of earthworms found in each sample point, i.e. per monolith), and other invertebrates (number of each order/higher taxa of other invertebrates found in each sample point, i.e. per monolith, per 3 Winklers, and per 3 pitfalls).

The raw data enable us to calculate total density of the group. The total density pooled per land use type, for instance, makes it possible to do group-to-group comparison of their densities (relative density, relative abundance) among land use types. The raw data in Table 3 gives total density values of 182, 72, 35, 38; 33, 26, and 8 of termite individuals, respectively for the following land use types: undisturbed forest (FLI), disturbed forest (FI), shrub, polyculture coffee (TBLI), monoculture coffee (TBI), food crop (CBLI), and vegetable crop (CBI). Assuming that four similar data are also available (i.e. total density of ants, beetles, earthworms, and other invertebrates, respectively pooled per land use type in the same sequence), then the total density of termites contributes to 22.1, 11.9, 8.2, 11.3, 13.1, 13.1, and 6.6% of the grand total density of below-ground macrofauna in the corresponding land use types. Figure 3 illustrates the relative abundance of the five major groups of below-ground macrofauna. Information that can be drawn from that figure is as follows. Of the five major below-ground macrofauna groups, the relative abundances of termites, earthworms, and other invertebrates decrease in land use types with higher intensification. In contrast, the relative abundance of ants increases and that of beetles remains stable with increasing land use intensification.

Biomass can also be tabulated similar to Table 3 above. For earthworms, this is convenient because biomass records can easily be gained for each species per each sample point. For other groups, such as ants, the biomass cannot always be recorded directly due to small size of some ant species.

However, a total biomass for each group (pooled per land use type) can be obtained. As a result, not only the total biomass but also relative biomass of each macrofauna group can be compared (among land use types) and presented as in Figure 3.

The next step is to look at mean diversity and mean density of the macrofauna across the observed land use types, in group by group and/or method by method fashion. The mean values are taken per land use type (thus averaged from a number of sample points taken per land use type). Overall analysis of variance (F-test) is performed to make sure if there is any significant difference among samples (land use types). If there is, then mean comparison among land use types is performed. Using the least significant difference (LSD test), for example, is aimed at observing the difference of any pair of samples (land use types). For the monolith data, the means are compared among the observed land use types (F-table value with 6 and 28 degrees of freedom) and among monolith layers (litter versus 0"10 cm versus 10"20 cm versus 20"30 cm, F-table value with 3 and 16 degrees of freedom) (Swift and Bignell, 2001) while for the other data comparisons are made among land use types only. Figure 4 shows the mean diversity and the mean density of termites across seven land use types (data from Table 3).

It is also convenient to express diversity with an index, for example the most common Shannon-Weaver diversity index (H') (Price, 1980), as follows:

$$H' = - \Sigma p_i \log_e p_i$$

where p_i is the proportion of the ith taxa in the total sample. The taxa can mean species (for termites or earthworms), genus (for ants), family/subfamily (for beetles), or order (for the other invertebrates). The "total sample" term can be obtained from two levels of data pooling. To do comparison among land use types, data can either be pooled at the sample point level resulting in sample point total or pooling is done at the land use level which gives a land use total. The former makes the statistical comparison possible (taking into account the error term within land use as indicated by error bars in the histogram presentation) while for the later comparison is possible without considering variability within land uses. Figure 5 shows Shannon-Weaver index of termites across seven land use types with both levels of data pooling.

Then, there is a need to present the functional grouping of the data. Good taxonomic data should be tractable into ecological functions. This was

based on the assumption that, as part of an ecosystem, each biotic existence (i.e. species in particular and any identified taxon in general) should contribute to certain function in the environment. One of the functions relates to its feeding habit. Knowing the abundance of plant feeders and predators, for instance, is very crucial for pest control. The same is true of soil feeders for soil processes/functions. In other words, feeding group is considered to be a very important functional group. A key (clue) is needed to find out the functional (feeding) group in the literature, and that key *is* the species (or identified taxon). Table 4 provides an example of the literature search result for termite functional (feeding) groups. In the case of termites, the four feeding groups (i.e. Group I, Group II, Group III, and Group IV) (Donovan *et al.*, 2001; Eggleton *et al.*, 2002) can be simplified into two (Gathorne-Hardy and Jones, 2000; Gathorne-Hardy *et al.*, 2001; Jones *et al.*, 2003), i.e. the wood feeders (Group I + Group II) and soil feeders (Group III + Group IV). Figure 6 illustrates the distribution of the soil and wood-feeding termites (data in Table 3 are rearranged in terms of soil feeders and wood feeders as in Table 4) across seven land use types. Similar approach can be used for presenting the functional grouping of beetles (herbivore-predator-scavenger-fungivore scheme) (Chung *et al.*, 2000; Hammond, 1990) or of earthworms (anecic-epigeic-endogeic, native-exotic scheme) (Fragoso *et al.*, 1997).

It is also important to show the relationship between the biological (taxonomic) variables and the ecological (soil) variables The resulting coefficient of determination (R^2) and regression slope (b) are calculated and test of significance for slope can be determined using PROC REG procedure (SAS Institute Inc., 1989). As an example, the diversity (number of species) and density (number of encounters) of termites (all termites combined, wood-feeding termites only, or soil-feeding termites only) can be regressed with soil pH, soil bulk density, soil carbon, soil nitrogen, leaf litter, or others (as in Jones *et al.*, 2003). Of course not all correlations are significant; but from those facts, a sound scientific explanation can be given (increase in bulk density or soil compaction due to mechanized plowing reduces density of soil-feeding termites) and a fine technical recommendation can be offered (leaving dead wood on the ground after forest cutting to supply resources for all termites). Methods to collect soil characteristics (variables) are described in Chapter V (this book).

Table 3. An example of raw data indicating the number of termites found per 25 x 25 x 30 cm monolith (hypothetical data)*

No	Species names	Undisturbed forest (FLI)					Disturbed forest (FLI)					Shrub				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	*Bulbitermes constrictus*					2		1								
2	*Bulbitermes singaporiensis*							2								
3	*Bulbitermes sp. A*						3									
4	*Hypotermes xenotermitis*															
5	*Labritermes sp. A*	3	1	3		2				1		1				
6	*Longipeditermes longipes*	7				3	7									
7	*Macrotermes ahmadi*	2	9	2	3	4	2	1	7	3	1	1				
8	*Macrotermes gilvus*	1		1	4			2		1		1	2		3	1
9	*Macrotermes malaccensis*	2	6	3	2	3	1		2	1			1	1		3
10	*Mirocapritermes connectens*	2	2	3	4	1			2		1		1			
11	*Nasutitermes havilandi*	1	1	1		2									1	
12	*Nasutitermes sp. B*							1		1	2					1
13	*Odontotermes denticulatus*	1	1	2	3	4		1			1					
14	*Odontotermes sarawakensis*															
15	*Odontotermes sp. H*											2		1		
16	*Parrhinotermes aequalis*									1			1		1	1
17	*Parrhinotermes sp. C*	1	2		1		3	2	1	1						
18	*Pericapritermes buitenzorgi*	2	1	2	2	1	1			1		1		1		
19	*Pericapritermes dolichocephalus*	1	1	2	4	1		2								
20	*Pericapritermes nitobei*	2	1	2	1	1	1		1							
21	*Pericapritermes sp. A*	3	4	2	3	5		2								
22	*Pericapritermes speciosus*	2	1	2	5	2	1		2							
23	*Procapritermes sp. C*	3	2	2	1	1				1			1	2		1
24	*Schedorhinotermes brevialatus*	2	1	2	1											
25	*Schedorhinotermes javanicus*		1		2	1	1	2			2					
26	*Schedorhinotermes malaccensis*														1	
27	*Schedorhinotermes mediocbscurus*										1				3	
28	*Schedorhinotermes sarawakensis*															
29	*Schedorhinotermes tarakanensis*															
30	*Termes comis*	5	4	3	2	1					2					

*) Assuming observation is done on 7 land use types (Evizal and Budidarsono, 2005), 5 sample points (i.e. 5 monoliths) per land use type are selected randomly from eligible gridded points in a research site (based on 200 x 200 m between-point grids in the IKONOS image map, ground-checked) after Afandi *et al.* (2005); empty cells = zero termites.

Continued data of Table 3 from page 18 (left).

No	Polyculture coffee (TBLI)					Monoculture coffee (TBi)					Food crop (CBLI)					Vegetable crop (CBI)				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1																				
2																				
3																				
4						2														
5																				
6																				
7																				
8		3																		
9			2		1		2			1	1	2	2	5	1	2		1	2	
10																				
11																				
12																				
13	2		1	1	1		4	3	2					2						
14	3		2	3		4	1	1	4	2	1			1			1			2
15	2		1			1		2	1	1	1	1		1						
16																				
17																				
18		1																		
19																				
20				1																
21		1																		
22																				
23																				
24																				
25																				
26													3							
27								2				4								
28												1								
29																				
30																				

*) Assuming observation is done on 7 land use types (Evizal and Budidarsono, 2005), 5 sample points (i.e. 5 monoliths) per land use type are selected randomly from eligible gridded points in a research site (based on 200 x 200 m between-point grids in the IKONOS image map, ground-checked) after Afandi *et al.* (2005); empty cells = zero termites.

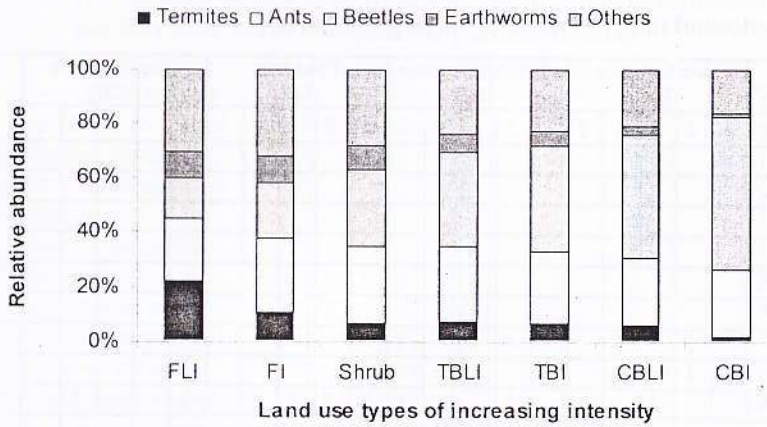

Figure 3. The relative abundance of five major groups of below ground invertebrates across seven land use types.

(FLI = undisturbed forest, FI = disturbed forest, TBI = monoculture coffee, TBLI = polyculture coffee, CBLI = food crop, CBI = vegetable crop)

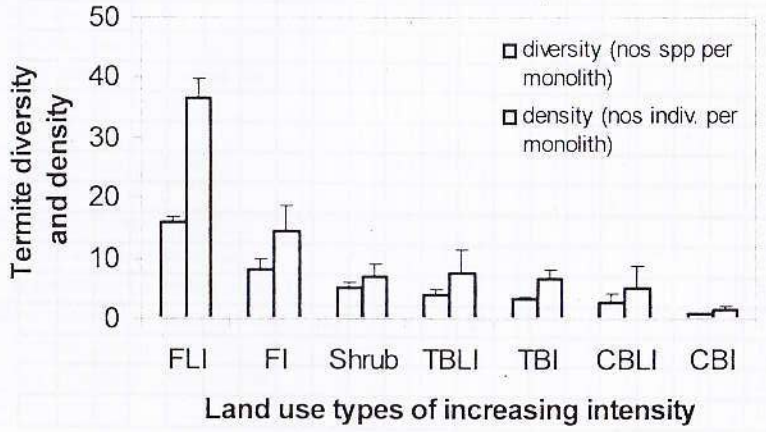

Figure 4. The diversity and density of termites across seven land use types.

(FLI = undisturbed forest, FI = disturbed forest, TBI = monoculture coffee, TBLI = polyculture coffee, CBLI = food crop, CBI = vegetable crop). Bar is standard deviation.

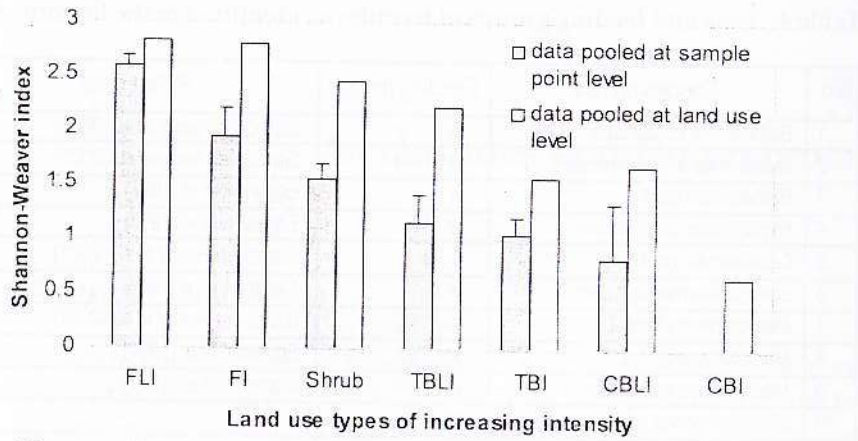

Figure 5. Shannon-Weaver diversity index of termites across seven land use types.

(FLI = undisturbed forest, FI = disturbed forest, TBI = monoculture coffee, TBLI = polyculture coffee, CBLI = food crop, CBI = vegetable crop). Note data pooled at sample point level with standard deviation (bars) and those pooled at land use level without the bars

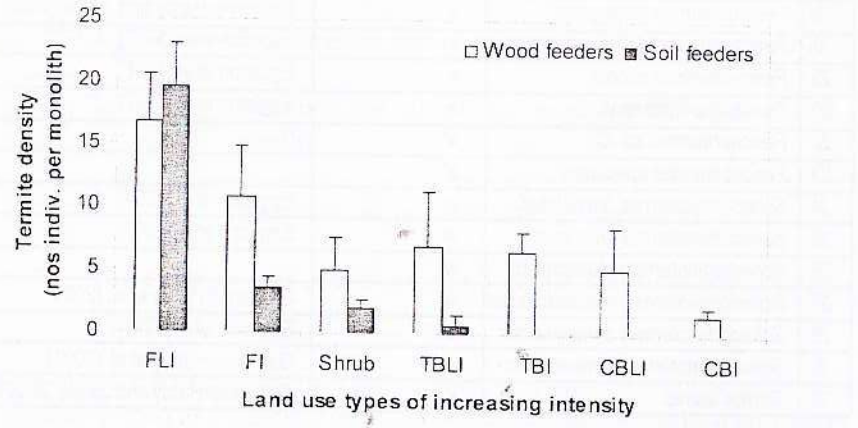

Figure 6. The density of two feeding groups of termites across seven land use types.

(FLI = undisturbed forest, FI = disturbed forest, TBI = monoculture coffee, TBLI = polyculture coffee, CBLI = food crop, CBI = vegetable crop), bar is standard deviation

Table 4. Taxa and feeding groups of termites as identified in the literatures

No.	Species names	Feeding groups	References
1	*Bulbitermes constrictus*	w / l / g	Gathorne-Hardy *et al.* (2001)
2	*Bulbitermes singaporiensis*	w / l / g	Gathorne-Hardy *et al.* (2001)
3	*Bulbitermes sp. A*	w	Eggleton *et al.*(1997)
4	*Hypotermes xenotermitis*	w / f	Eggleton *et al.*(1997)
5	*Labritermes sp. A*	s / w	Gathorne-Hardy *et al.* (2001)
6	*Longipeditermes longipes*	w / l	Eggleton *et al.*(1997)
7	*Macrotermes ahmadi*	w / l / g	Gathorne-Hardy *et al.* (2001)
8	*Macrotermes gilvus*	w / l / f	Eggleton *et al.*(1997)
9	*Macrotermes malaccensis*	w / l / f	Eggleton *et al.*(1997)
10	*Mirocapritermes connectens*	s*	-
11	*Nasutitermes havilandi*	w**	-
12	*Nasutitermes sp. B*	w	Eggleton *et al.*(1997)
13	*Odontotermes denticulatus*	w**	-
14	*Odontotermes sarawakensis*	w / f	Eggleton *et al.*(1997)
15	*Odontotermes sp. H*	w**	-
16	*Parrhinotermes aequalis*	w	Eggleton *et al.*(1997)
17	*Parrhinotermes sp. C*	w**	-
18	*Pericapritermes buitenzorgi*	s	Gathorne-Hardy and Jones (2000)
19	*Pericapritermes dolichocephalus*	s	Eggleton *et al.*(1997)
20	*Pericapritermes nitobei*	s	Eggleton *et al.*(1997)
21	*Pericapritermes sp. A*	s	Eggleton *et al.*(1997)
22	*Pericapritermes sp. C*	s*	-
23	*Pericapritermes speciosus*	s*	-
24	*Schedorhinotermes brevialatus*	w	Eggleton *et al.*(1997)
25	*Schedorhinotermes javanicus*	w	Eggleton *et al.*(1997)
26	*Schedorhinotermes malaccensis*	w	-
27	*Schedorhinotermes mediocobscurus*	w	Gathorne-Hardy *et al.* (2001)
28	*Schedorhinotermes sarawakensis*	w	Eggleton *et al.*(1997)
29	*Schedorhinotermes tarakanensis*	w	Gathorne-Hardy *et al.* (2001)
30	*Termes comis*	s / w	Gathorne-Hardy and Jones (2000)

Notes: w = feed on wood, s = feed on soil, s/w = feed on soil-wood interface fragment, l = feed on leaf litter, g = feed on grass, f = feed on fungus, * = included in the Capritermes group with dark gut content (interpreted as soil feeder), ** = with light gut content and affinity to Nasutitermes or Parrhinotermes group (interpreted as wood feeder), w/l/g, w/l/f, and w/f are pooled into w group while s/w is pooled into s group.

3
Nematodes and Collembola

3.1 Nematode Sampling

Nematodes are sampled from soil cores. Twelve soil cores (sub-samples) are obtained at each sampling point. The position of sub samples is in 3-m and 6-m rings, respectively (see Figure 1 and Figure 7), with the monolith as a center point. In the 3-m ring, four soil cores are collected at the north, east, south, and west positions. In the 6-m ring, eight sub samples are taken at the north, northeast, east, southeast, south, southwest, west, and northwest positions. A compass is used to locate the correct cardinal position, and a measuring tape is used to mark the correct distance of the sampling points from the monolith. The determined point is marked with a bamboo stick before starting digging.

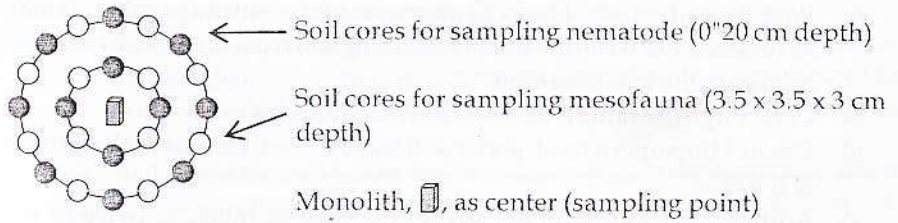

Figure 7. The arrangement of soil cores in 3-m and 6-m rings for sampling nematode and mesofauna.

After all litters are removed from the surface, soil sample is collected with a small spade (*cetok*) at 0"20 cm depth. When condition permits, a soil borer should be used to collect the soil. Soil from 12 sub-samples is combined into a 5-kg capacity plastic bag. From the composite, a ½ kg of soil is taken for general nematode analysis and ½ kg for entomophatogenic nematode analysis (if needed). The soil sample is collected in a plastic bag and stored temporarily in a shade to avoid unnecessary exposure to direct sunlight.

Plastic bag filled with soil sample for entomophatogenic nematode should be pierced with a needle or pointed object to prevent moisture retention or condensation inside the bag.

Soil samples collected from the field are then packed into cartoon box before being transported with a ground vehicle into the laboratory. Upon arrival in the laboratory, soil sample is extracted as soon as possible; otherwise it should be stored at 4 °C for future use.

3.2 General Nematode Extraction and Identification

Nematode is extracted or isolated by using a modified method of flotation-centrifugation with sucrose solution (Jenkins, 1964), with the procedure as follows:

1) Put 300 cc soil samples into a 5-l capacity plastic bucket
2) Add 2 liter of tap water, stir thoroughly and squeeze it for 30 seconds
3) Let the soil suspension standing for 2 min before filtered gradually using 1 mm and 53 µm (200 mesh) sieves and discard soil sediment at the bottom of bucket
4) Pour the soil collected from 53 µm sieving into centrifuge tubes. It may be necessary to wash away the remaining soil from the sieve in order to minimize the loss of sample
5) Centrifuge the sample at 3500 rpm for 5 minutes
6) Discard the supernatant, and it will leave the soil residue at the bottom of the tube
7) Add sucrose solution (500 g/l) to the tube as much as twice of the height of soil residue and stir thoroughly
8) Centrifuge the mixture at 1000 rpm for 1"2 minutes
9) Collect the supernatant containing nematodes in sucrose solution by sieving it at 38 µm (400 mesh) and discard the soil
10) Using a spray bottle, wash sucrose solution (sieved supernatant) with water and collect aliquot in a suspension bottle

Killing, fixation, and counting nematode

Nematode inside the suspension bottle is killed with warm water (50 °C) for less than 1 min. Bottle containing 25 ml of nematode suspension, with thermometer inside, is immersed in boiling water. Upon reaching 50 °C, the bottle is taken out and cooled. After cooling, Golden solution is added (8 part formalin + 2 part glycerine + 90 part distilled water), final 3% of formalin (Hopper, 1970) up to 40-ml volume. After a while, the volume is reduced into 15 ml by carefully pipetting from the upper part of the solution to minimize the loss of the sedimented nematode at the bottom of the bottle. Transfer 3 ml of nematode suspension into a 5-cm Petri dish graded at 1 x 1 cm. Count the nematodes under stereo a binocular microscope at 40 X magnification. Counting should be done three times, and the population of nematode is the average of the three-counting times 5.

Nematode identification

For nematode identification, we need to prepare a permanent specimen. The specimen is prepared according to the modified method of Seinhorst (1959), following the process of infiltrating glycerine into nematode's body. The process of glycerine infiltration is as follows:

1) Pour 3 ml of nematode suspension into a 5-cm Petri dish
2) Add 7 ml Seinhorst I solution (20 part 96% alcohol + 2 part glycerine + 78 part distilled water) and store the Petri dish in a desiccator filled with saturated alcohol (96%) at 43 °C for a night (desiccator is placed in an oven)
3) In the next morning, take the Petri dish out from the desiccator and put into the oven at 43 °C for four hours, enough to reduce its volume into a half
4) Add Seinhorst II solution (95 part 96% alcohol + 5 part glycerine) up to the volume of 10 ml, and store again in the desiccator for a night
5) Repeat the process three times
6) Then, put the Petri dish containing the suspension of nematode into the oven at 43 °C for 48 hours. The nematode is ready to be made as permanent specimen.

The nematode specimen is made on 5 x 2 cm object glass under a stereo binocular microscope at 40 X magnification. First, a drop of pure glycerine is placed on a center of the glass and three pieces of glass wool are put over the glycerine to make a triangular form. Second, nematodes are scooped

carefully from the suspension and poured into the object glass, in such a good position at the innermost of the glass wool triangle. Third, an Arabic gum is spread over the nematode sample, and then a cover glass is placed on the top of it.

Approximately 100 nematode specimens are taken from each sample for identification. The nematode is identified until genus-taxa category under a compound microscope at 400 - 1000 X magnification. Nematode specimens are identified following Goodey (1963), Andrassy (1983), May and Lyon (1975) and Siddiqi (1985). As of genus level, nematodes are generally classified into five functional groups: bacteriovore-, fungivore-, plant parasitic-, omnivorous-, and predator-nematodes (Yeates *et al.*, 1993).

3.3 Collembola Sampling and Identification

Collembola are mainly sampled from soil cores and optionally collected from pit-falls. Twelve sub-samples are obtained from one sampling point. The position of sub-samples is arranged circularly around the center point; 4 sub-samples spaced equally in the 3-m ring and 8 sub-samples from 6-m ring (Figure 1 and Figure 7). Soil sample is collected from 3.5 x 3.5 x 3 (depth) cm by using a small spade with certain volume. Soil from 12 sub- samples is then placed on a 5-kg plastic bag as a composite.

The composite soil sample of is then divided into 10 plastic bags averaging of 1 kg, labeled, and all bags are transferred into a cloth bag, with the dimension of 30 x 35 cm, to prevent the death of collembola. Cloth bags have been very efficient for temporary storing and transporting sample for a longer distance and time because the cloths allow air to circulate and prevent unwanted rise in temperature. A plastic-bag should not be used since it could substantially increase the soil temperature and may lead to collembola's death. Also, unnecessary exposure to direct sunlight must be avoided during sample bagging in the field. Upon arrival in the base-camp, all cloth bags are arranged according to their sample sites. Soil samples are then packed in a cartoon box pre-layered with paper to maintain moisture stability inside the box. Samples are then ready to be transported, preferable using an air-conditioned vehicle, into the laboratory for identification.

Upon arrival in the laboratory, the soil is immediately transferred into a Berlese funnel, i.e. on a 2 x 2-mm sieve (13 mesh) wire platform inside the coned-aluminum funnel (Figure 8). A light bulb of 10 watt is often set above soil samples and the lid on top is closed to prevent entry of other insects. With the presence of light, incubation time (amount of time needed to extract the faunas from soil) takes 4"5 days. In some cases, heating/ drying process could be done simply using room temperature; with this process the incubation time takes 7"10 days, depending on initial soil moisture condition.

The use of light bulb might attract nocturnal insect especially if Berlese funnel is not properly closed or soil might be dried too soon due to increasing temperature. The intruder insect could overestimate the number of faunas while the fast drying soil might cause the death of the faunas. Using room temperature is relatively safe but the top of the funnel must also be tightly closed.

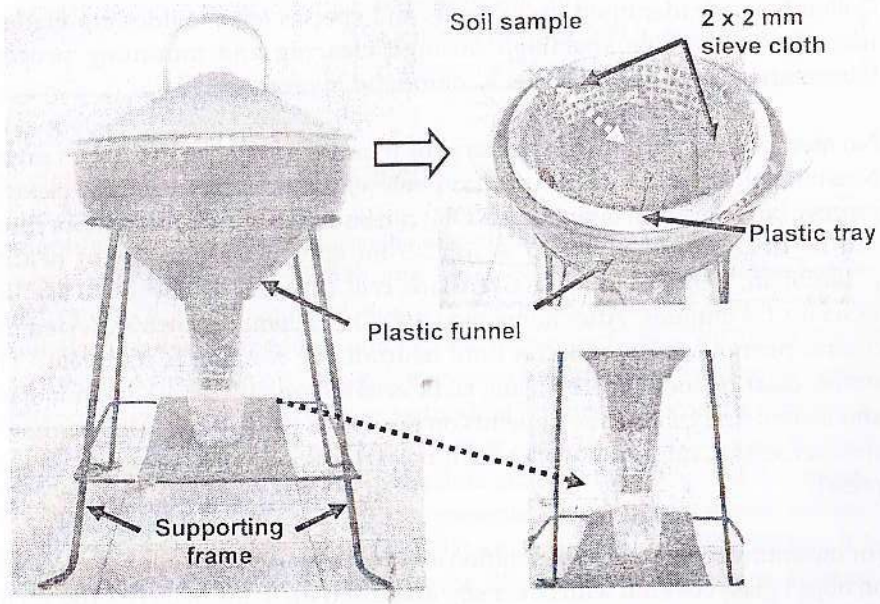

Figure 8. A modified Berlese funnel made from plastic material (left) and close up view of sample inside the tray and a collecting jar (right).

The basic principle of Berlese's funnel is that soil organisms will avoid the increase temperature or dryness in the soil. A soil or leaf sample is placed in the removable upper part of the funnel. Heat or light from the lamp creates a temperature gradient in the soil sample; the upper part of the soil is warmer than the lower one. This condition stimulates the downward movement of soil arthropods, and similar organism, into deeper layers to find lower temperature/ more humid environment, and finally through the gauze (wire mesh) to a receiver container attached to the base of the funnel. The position of the lamp is adjustable to enable the temperature of the soil to be raised gradually, thus preventing the slower moving species from becoming trapped in hard dry cakes of soil. The receiver, usually a jar or a bottle, is filled with 96% ethanol for preservation and harvested at 4"5 days intervals.

Specimen clearing and mounting processes

Collembola are identified up to genus and species level. Slides are made to identify Collembola specimen through clearing and mounting process. Observation is conducted under a compound microscope.

No methods are entirely satisfactory for mounting (Grenslade *et al.* in press). Neisbitt solution (see its composition below) is commonly used for clearing process, but potassium hydroxide (KOH) can be used as an alternative. Specimen can be cleared with KOH 15 % for 2-5 minutes, with or without heating (Ubaidillah, 1999), meanwhile Grenslade *et al.* (in press) proposed to use 10% KOH for 1"5 minutes. After immersion in KOH, collembola are transferred into chloral phenol for few minutes until neutralizing reaction is complete. The simple clearing method is by using lactic acid where collembola are immersed and heated for certain time depends on the size of collembola. This method is cheaper, easier, safer, and work well for clearing specimens (Grenslade *et al.* in press).

For mounting process, Berlese solution is very suitable. Specimens are mounted on object glass covered with cover slip and dried up in the oven (70-100°C) for 2"3 days before observed under microscope. Other solution, such as Hoyer medium might also be used for mounting process (Table 5).

Table 5. The chemical composition for clearing and mounting specimen

1. Clearing specimen	Composition
Neisbitt solution	a. 25 ml distilled water , b. 40 g chloral hydrates, and c. 2.5 ml of 1N HCl.
10 % KOH or NaOH	
2. Mounting specimen	
Berlese solution	a. 20 ml distilled water, b. 15 g Arabic gum, c. 50 g chloral hydrates, d. 5 ml glycerine, and e. 5 ml glacial acetic acid
Hoyer medium	a. distilled water 50 ml, b. gum Arabic 30 g, c. chloral hydrate 200 g, and d. glycerine 16 ml

Specimen identification and data analyses

The identification and classification of collembola are based on Grenslade *et al.* (in press), Yosii (1981, 1982a, 1982b, and 1983). Number of individuals of each order from each site is counted to study the abundance. Comparison of collembolan species composition among land uses is performed by hierarchical analysis using Average linkage of agglomerative clustering based on transformed abundance numbers. The number of individual is transformed using following equation: $x'=1\log10(x+1)$. Transformation is used to avoid the risk of over emphasizing of dominant species in the data analysis (Ludwig and Reynolds, 1988). The species diversity could be presented by using Shannon Wiener Diversity (H) and Simpson's Diversity (1 D), i.e. with Program DIVERS Ver. 5.1 from Ecological Methodology Package for Windows. The Shannon Wiener index is particularly sensitive to the abundance of rare species, whereas Simpson's Index is more sensitive to changes in the more abundant species than rare species (Krebs, 1989). Simpson's index is used to calculate the distribution of abundance each species (equitability).

4
Microbes

4.1 Soil Cores for Sampling Microbes

Soil cores for sampling microbes are taken similarly to nematodes' sampling. The position of twelve sub-samples is depicted in Figure 9.

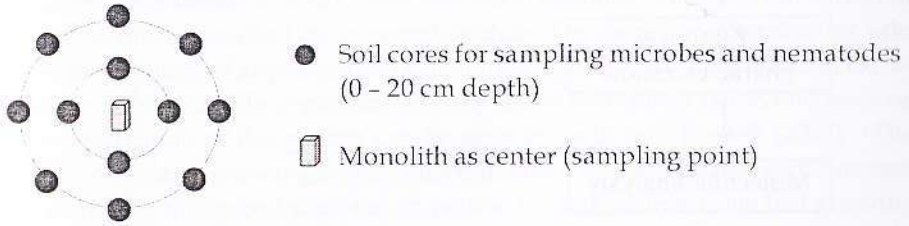

Figure 9. The arrangement of soil cores in 3-m and 6-m rings for sampling microbes

Litter must be removed just before sampling time. All sampling materials must be flamed before and after sampling at each point to avoid any possible introduction of exotic microbes. Twelve sub-samples are obtained from 0"20 cm soil depth and collected to make one composite sample. The amount of composite depends on the number of functional group to be studied. For example, a 2.5-kg bulk is sufficient for studying five functional groups of microbes or 0.5 kg of soil for each group. Similarly, small auger cores of about 50 g each to a depth of 20 cm in each 12 sub-samples is enough to get 500 g of soil sample. Samples are put in plastic bags, labeled, and then transported into the laboratory with an air-conditioned vehicle.

4.2 Legume Nodulating Bacteria (LNB)

The identification of legume nodulating bacteria (LNB) or for simplicity just referred as rhizobia requires tedious work involving the use of molecular marker. The diagrammatic procedures of rhizobial research are shown in Figure 10 and Figure 11.

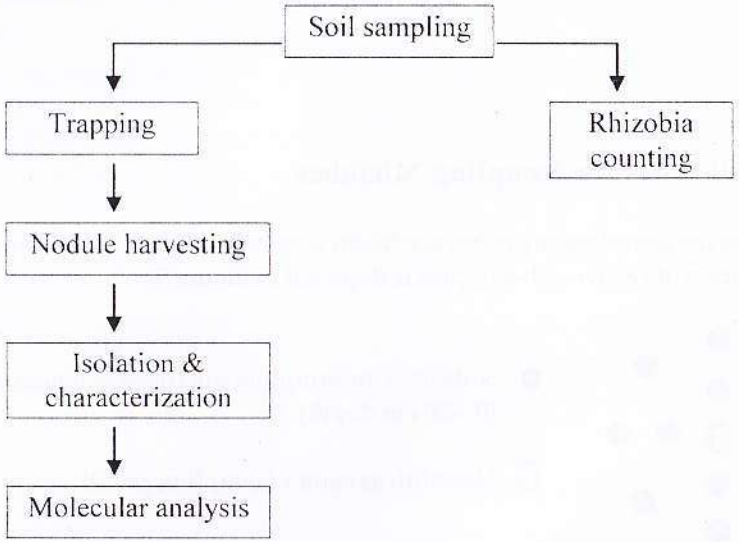

Figure 10. Chart of rhizobial research.

Rhizobia counting

Materials for rhizobia counting are 1-ml and 5-ml pipettes, diluent solution, 100-ml and 125-ml Erlenmeyer flasks, autoclave, test tubes (200 x 30 mm), racks for tubes, agar, chemicals for nutrient solution ($CaHPO_4$, K_2HPO_4, $MgSO_4.7H_2O$, NaCl, H_3BO_3, $MnSO_4.H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $NaMoO_4.2H_2O$, $FeCl_3$), seeds of host plants (siratro = *Macroptilium atropurpureum*), and growth room.

Rhizobia counting are based on the plant infection method. Soil samples are submitted to a four fold serial dilution before host plants are inoculated. Each dilution is replicated three times. Plants are grown under controlled environmental conditions in growth room (Vincent, 1970), and examined for nodule formation after 15 days. Populations of rhizobia are estimated with the Most Probable Number method (Bennett and Woomer, 1990).

Rhizobia isolation and identification

Materials for rhizobia isolation are alcohol, $HgCl_2$, sterile water, forceps, inoculation loop, isolation needle, scalpel, Bunsen burner, sterile Petri dishes, sterile Pasteur pipettes, sterile Erlenmeyer flasks, refrigerator, autoclave, chemicals for YMA with Congo red indicator (CR YMA), YMA with bromthymol blue indicator (BTB YMA), mannitol, K_2HPO_4, $MnSO_4.4H_2O$, NaCl, yeast extract, distilled water, agar, Congo red, bromthymol blue.

Rhizobia are isolated from the nodules formed in the trap plants grown in the soil samples from each sampling point. At most seven nodules from each pot are harvested. The first step is to surface sterilize the nodules by a brief immersion in 95% alcohol, followed a longer immersion up to 3 4 minutes in $HgCl_2$ and washing in several rinses of sterile water (Vincent, 1970). Each nodule is then crushed in a few drops of sterile water, using forceps, and a loopfull of this suspension is streaked onto Congo red YMA medium. At most four different single colonies are picked up from each nodule. The characters observed include growth rate (time of appearance of isolated colonies = TAIC), the extent of extracellular polysacharide deposition, colony shape and colony color, and texture. The description of the genera can be seen in Swift and Bignell (2001). The ability of isolates growing on bromthymol blue YMA to produce acid reaction by changing the color from blue to yellow is used to determine fast growing rhizobia.

Molecular analysis of rhizobia

The genetic diversity of the isolates is assessed by the DNA fingerprinting of the repetitive extragenic palindromic sequences (REP-PCR) by using 16S-rRNA gene with the *polymerase chain reaction* (PCR). The electrophoretic patterns of the PCR products are specific for each strain. The analysis of genetic diversity is using *amplified ribosomal DNA restriction analysis* (ARDRA) technique. The molecular method for identifying rhizobia is shown in Figure 11.

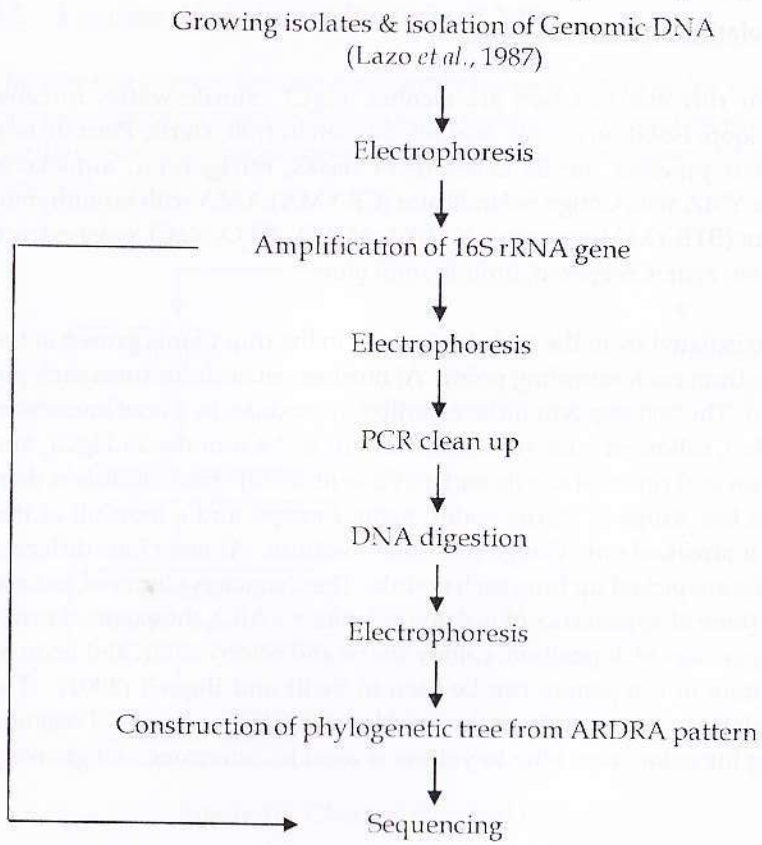

Figure 11. Steps of molecular analysis of rhizobia.

Growth of rhizobia and genomic DNA isolation. The rhizobial isolates are grown in 5 ml yeast mannitol broth at 28 °C for 3 days. The genomic DNAs are isolated by the method described in Lazo *et al.* (1987).

Electrophoresis. The first electrophoresis is done by the method described in Sambrook and Russell (2001) to assure that the DNAs are well-isolated. The second electrophoresis is done to assure that the 16S rRNA genes are well-amplified. The third electrophoresis is done to assure that the PCR products are well digested. The gels are observed and documented by using ChemiDoc™ EQ (Biorad, Richmond, CA).

PCR amplification of the 16S rRNA gene. The 16S rRNA gene is amplified with primers 16Sa (5' - CGCTGGCGGCAGGCTT AACA - 3') dan 16Sb (5' - CCAGCCGCAGGTTCCCCT - 3') (van Berkum and Fuhrmann, 2000). The PCR amplifications are done using PTM-100™ Programmable Thermal Controller (MJ Research Inc., USA) programmed as follows: a pre-start at 95 °C for 3 min, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, and a final extension at 72 °C for 3 min.

PCR clean up. The PCR products are purified using QIAQuick Spin Column (Qiagen).

DNA Digestion. The purified PCR products are digested with restriction enzymes *Rsa* I, *Hae* III and *Hha* I (New England, Biolabs Inc.).

ARDRA analysis. The biner data of the digested PCR products are combined for each sample and analyzed using Treecon Software Copy right Yves van de Peer (Belgium) to construct the phylogenetic tree.

Sequencing. Sequencing is done by using the Beckman Coulter CEQ™ 2000 Sequencer. The DNA sequences are then compared to the European Bioinformatics Institute (EBI) databases sequence (http://www.ebi.ac.uk).

4.3 Arbuscular Mycorrhizal Fungi (AMF)

Multiplication of AMF

The population of indigenous AM fungi can be rescued by a trap culture technique as described in Setiadi and Noor (2004). Trap culture could be made on drainage 300 cc plastic cups, using washed zeolite as a medium and two different plants as a host, i.e. *Sorghum vulgare* (sorghum) and *Pueraria javanica* (kudzu). Hundred fifty cc of soil sample are filled into the plastic cup on layered of zeolite.

Two surface-sterilized and pre-germinated seeds of sorghum or kudzu are sowed on the filled plastic cup, placed in the green house, and maintained for 3"4 months for sporulation. The plants are watered daily and fertilized with 10 ml per cup of red hyponex (1 g in 5 l water) every week. After 3"4 months, the AMF spore are harvested and used for taxa identification.

AMF extraction and identification

Spore extraction and isolation are carried out using the sieving and decanting method with a slight modification (Setiadi and Noor, 2004). Soil samples (50 g) are suspended in 300 ml tap water and placed in a liquidizer, and blended for two successive periods of 2 sec prior to wet sieving. Blending is carried out to release the spores of certain species of AMF which form inside roots e.g. *Glomus manihotis*. The suspensions are then left standing for 1 min to allow larger particles to sediment, before being decanted through a series of soil sieves (250, 125, and 45 µm mesh size) and washed thoroughly several times with tap water. The sieved materials on the finest sieve are then transferred with approximately 100 ml water into a 500-ml beaker, stirred with a glass spatula, and decanted as a 20"25 ml aliquot into 50-ml centrifuge tubes. Twenty five to thirty ml of 60% (w/v) sucrose solution (household sugar) are then injected into the bottom of each tube using a 50 ml syringe to which a plastic pipe (15"20 cm long, with an inner diameter of 0.5 cm) has been attached. The tubes and contents are then centrifuged at 2900"3000 rpm for 2 min in an MSE bench Centaur centrifuge.

Spores are recovered by gently sucking up the suspension using the same syringe from just above the water/sucrose interface and ejecting it into a clean sieve (45 µm mesh). The sieved contents are then rinsed 2 3 times with water, before being transferred in water to a Petri dish for observation under a dissecting microscope at 40 X magnification. Spores or sporocarps which are morphologically similar are "properly" grouped, separated, and transferred into 3-cm Petri dishes using a Pi pump micropipette, ready for identification or for culture purposes (Setiadi and Noor, 2004). AMF identification for genera level is made according to the "Manual for identification of VAM fungi (Schenck and Perez, 1990).

4.4 Saprophytic/Decomposing Fungi (SDF)

Soil sampling for microbes is previously described. Soil samples should be transported directly to the laboratory and the isolation of cellulolityc and lignin degrading fungi is conducted at once. Water holding capacity of the soil samples is determined directly. Soil samples are then pre incubated in the laboratory for 7 days at field capacity. The pre incubated soil samples are then used to isolate cellulolityc and lignin degrading fungi. One tenth soil dilution series is used in three replicates by using sterile physiological solution (8.5 g NaCl L^{-1} water).

The methods for identifying lignin and cellulose degraders are based on Verstraete (1981) and von Mersi and Schinner (1995), while general reference of soil fungi can be found in Gilman (1957), Raper and Fennell (1965), Booth (1971) and von Arx (1981).

Lignin degraders

Lignin is a highly complex molecule and the structure differs from genera to species. Lignin is highly recalcitrant to degradation. Normally lignin has phenyl propane structure. Some fungi belonging to the class of Basidiomycetes can degrade lignin by producing certain enzymes. Those fungi are *Agaricus, Armillaria, Claveria, Clitopybe, Coprinus, Fomes, Ganoderma, Pleurotus, Polyporus* etc.

Some enzymes are known to involve in lignin degradation (von Mersi and Schinner, 1995). For example, phenol oxydase oxidizes aromatic compounds or structure containing 1 to 2 hydroxyl groups, laccase oxidizes aromatic compounds with more than one hydroxyl groups, and peroxydase oxidizes aromatic molecules but with the presence of hydrogen peroxide.

Isolation of lignin degrading fungi

Materials needed for extracting lignin degrading fungi are malt extract tannic acid agar medium (Table 6), pH meter, conical flask, Petri plates, pipettes, autoclave, laminar flow, Bunsen burner, 70% ethyl alcohol, glass marker, soil samples, and distilled water.

The procedure for the isolation of lignin degrading fungi is as follows:

1) Switch on UV lamp of the laminar chamber for a period of 15 minutes followed by a dark period of 15 minutes
2) Open the laminar door along with air blow and disinfect working surface of the laminar flow and spray with 70% ethyl alcohol
3) Transfer 10 g of soil sample to 90 ml sterilized physiological solution and shake vigorously for 5 minutes (10^{-1} dilution)
4) Transfer 1 ml of aliquot from soil extract (10^{-1} dilution) to 9 ml sterilized physiological solution making it 10^{-2} dilution, similarly transfer 1 ml from the 9 ml physiological solution to 9 ml sterilized physiological solution making it 10^{-3} dilution, similarly follow till 10^{-5} dilutions

5) Transfer 1 ml from dilutions 10^{-3}, 10^{-4} and 10^{-5} to sterilized Petri plates separately (start with 10^{-5}) and follow pour plate method by pouring molten malt extract tannic acid agar medium (33 mg of streptomycin sulphate per liter of the medium is added to avoid bacteria). It is necessary to have three replications for each dilution.
6) Incubate the inoculated Petri plates at 35 °C for 7 days

The lignin degrading fungi can be observed by the presence of diffusion zones or halo zones (appearance of light colored zones in comparison with dark colored surroundings). The suspected organisms are maintained on potato dextrose agar (PDA) (Petri plate for purification) and then grown on slants PDA. The fungi are then identified up to genus or species level.

Cellulose degraders

Cellulose is composed of glucose units linked by b-1,4 linkage. By the action of cellulase enzyme, cellulose is hydrolyzed into glucose units. The enzymes degrading cellulose are C_1, Cx (b glucanase) and b glycosidase. The first two enzymes are extra-cellular and the last one being intracellular. C_1 acts randomly on cellulose resulting in the formation of oligomers. Cx enzyme acts on oligomers resulting in the formation of dimers (cellobiose) which enters the cell; cellobiose is then attacked by b glucosidase to form glucose units. Some fungi involved in cellulose degradation are *Aspergillus, Alternaria, Chaetomium, Trichoderma, Fomes, Penicillium*, etc.

Isolation of cellulose degrading fungi

Materials required for the isolation are Dubo's cellulose agar medium (Table 6), pH meter, conical flasks, Petri plates, 9 ml and 90 ml sterilized physiological solutions, pipettes, autoclave, laminar flow chamber, Bunsen burner, 70% ethyl alcohol, glass marker, soil samples, and distilled water.

The procedure for the isolation of cellulose degrading fungi is as follows:
1) Switch on UV lamp of the laminar chamber for a period of 15 minutes followed by a dark period of 15 minutes
2) Open the laminar door along with air blow and disinfect working surface of the laminar flow and hands with 70% ethyl alcohol
3) Transfer 10 g of soil sample to 90 ml sterilized physiological solution to obtain a dilution of 10^{-1} and shake vigorously for 5 minutes (10^{-1} dilution)

4) Transfer 1 ml of aliquot from 10^{-1} dilution to 9 ml sterilized physiological solution making it 10^{-2} dilution, similarly transfer l ml from the 9 ml sterilized physiological solution making it 10^{-3} dilution, similarly follow till 10^{-5} dilutions
5) Transfer 1 ml from dilutions 10^{-3}, 10^{-4} and 10^{-5} to sterilized Petri plates separately and follow pour plate method by pouring molten Dubo's cellulose agar medium. It is necessary to have three replications of each dilution
6) Incubate the inoculated plates at 30 °C for a period of 7 days or till visible growth of the colonies is seen

The appearance of halo zones or yellowish color around the colonies indicates cellulase activity and such of those colonies are considered as cellulose degraders. The fungi are then identified up to genus or species level.

Table 6. Composition of the media used for isolation of lignin degrading fungi and cellulose degrading fungi (Dubo's Cellulose Agar Medium)

1. Media for lignin-degrading fungi	
Chemicals	Quantity
Malt extract	1.5 %
Tannic acid	0.5 %
Agar	2.0 %
pH	6.0 -6.5
2. Media for cellulose-degrading fungi (Dubo's Cellulose Agar Medium)	
Chemicals	Quantity
$NaNO_3$	0.5 g
K_2HPO_4	10 g
$MgSO_4 7H_2O$	0.5 g
KCl	0.5 g
$Fe_2(SO_4)_3 7H_2O$	trace
Cellulose	12 g
Agar	20 g
Distilled water	1000 m
pH	l6.0

4.5 Plant Pathogenic Fungi (PPF)

Although plant pathogenic fungi are many, this book is only focused on three main genera, namely *Pythium, Phytophthora* and *Rhizoctonia*.

Pythium spp.

The presence of *Pythium* spp. in soil samples is detected by using baiting technique. Soil samples (10 g) are placed in glass jars (8 cm diameter, 10 cm deep) and mixed with 20 ml distilled water. Five petals of rose are floated on the water in each jar. Jars are incubated at room temperature under diffused light on laboratory bench for 5 days after which time the petals are mounted in distilled water on a microscope slide and examined for the presence of sporangia of *Pythium* spp. Soil samples containing *Pythium* spp. are further analyzed for quantification of propagules using dilution plating of soil suspensions on P5ARP selective medium containing pimaricin, ampicillin, and rifampicin (Jeffers and Martin, 1986).

Phytophthora spp.

Soil samples are baited using the technique described for baiting of *Pythium* spp. Soil samples showing the presence of sporangia of *Phytophthora* spp are confirmed by baiting with cocoa pods as follows. Unripened, fully expanded cocoa pods (15-25 cm long) are used as baits. Moistened soil samples are inserted into a small hole (0.5 cm diameter, 0.5 cm deep) in the pods. The pods are then wrapped with moistened paper, incubated at room temperature on laboratory bench for 3-5 days. The presence of *Phytophthora* spp. is confirmed by the development of firm, dark brown lesions on the pods (Dance *et al.*, 1975; Darmono, 1997).

Rhizoctonia spp.

Soil samples are mixed thoroughly and sub samples of 50 g are wet-screened through a 0.3-mm mesh sieve (Weinhold, 1977). The material retained on the sieve is washed into a beaker containing water. The particles are evenly distributed on discs of filter paper (8 cm diameter) and each filter paper is inverted on the surface of agar plates (1% water agar or gallic acid selective media) (Ferris and Mitchell, 1976), agitated to dislodge and disperse the particles. The plates are incubated for 18 to 24 hr and suspected colonies are transferred to PDA for identification.

The determination of the total number of viable propagules of fungal pathogens requires either a method based on the number of colonies formed on an agar medium (known as plate count) or the recovery of pathogens on a series of dilution inoculated on a suitable host (plant infection count). The enumeration of microbes is described as follows:

Plate count

This method requires the preparation of a set of serial dilutions to provide 30 – 300 colonies at some step in the series. The procedures are as follows: One g of soil sample is dissolved into 99 ml sterile water to obtain 10^2 dilutions. To make 10^3 dilutions, one ml of the first dilution is put into 9 ml sterile water. The second step is repeated to obtain 10^4 and thereafter 10^5 dilutions. One ml of each dilution aliquot is poured into Petri dish and incubated at room temperature for a few days. The presence of colonies in plates containing 30 – 300 colonies are observed and counted. Counts are multiplied by the dilution factor to give the number of viable propagules per gram.

Modified Most Probable Number

Another method to enumerate the number of viable fungal pathogen is by recovery of pathogens within a series of dilutions on a suitable host as the bait. The procedure we called as Indirect Plant-segment Infection Count or M-MPN (modified most probable number). This method depends on the ability of some specific fungal genus to infect and grow on a specific host/bait.

The whole seedling or plant segments (fruits and tubers) could be used as the infection indicator or as the bait. The enumeration of viable propagules of soil-borne pathogen is conducted in four-fold dilutions prepared as follows. One g of soil sample is mixed with 3 g of sterilized sands to obtain 4^1 dilutions (mixture) of 4 g mixture. Two g of the 4^1 dilution is then inoculated to sterilized indicator plant segments at four different sites, i.e. 0.5 g mixture for each site. Next, one g of 4^1 soil mixture is given to another 3 g of sterilized sands to obtain 4^2 dilutions. One g of 4^2 soil mixture is further inoculated to other bait at four different sites. The step is repeated to obtain the next required dilutions.

The inoculated baits are incubated for a few days and examined for the appearing symptoms and signs of pathogens. The next step is isolating the associate microorganisms from each symptom site and identifying the pathogens. The following table shows a result of such experiment.

Table 7. An example of enumeration of microbes from Sumberjaya

Dilution	Replication	Microbes					
	1 2 3 4	Fusa*.	Botr.	Phyt.	Pyth.	Curv.	Rhiz.
4^1	+ + + +	+4	+2			+3	
4^2	+ + +	+2	+			+2	
4^3	+ + + +	+	+			+2	
4^4							
Total		7	4			4	

Source: Prasetyo and Aeny (2005) (unpublished data)

* *Fusa = Fusarium, Botr = Botryodiplodia, Phyt = Phytophthora, Pyth = Pythium, Curv = Curvularia, and Riz = Rhizoctonia*

The estimation of the count per gram soil is as follow:

$$X = \frac{m \cdot d}{v \cdot g}$$

Where m = likely number at dilution 1 in the series used for the entry in four-fold dilution (from Table 3.4 A in Vincent, 1970)
d = dilution represented by tube 1
v = volume (or weight) of the soil sample for inoculation at each site
g = weight of the soil sample

From the data in Table 7, we can count the estimated population of each pathogen found in the soil. The highest dilution level is 4 (s = 4); the total positive for Fusarium = 7, Botryodiplodia = 4 and Curvularia = 4. Therefore, the value of m for Fusarium = 3.2, Botryodiplodia = 1.1, and Curvularia = 1.1.

The estimated number of Fusarium (for example) per gram soil is:

$$X_{\text{fusarium}} = \frac{3.2 \times 4^1}{0.5 \times 1} = 25.6$$

5

Soil Physico-Chemistry Characteristics

Basically, there are three types of soil samples for various soil analyses:

1) Undisturbed soil sample, a soil sample taken using core (core sample), is used for determination of bulk density, pF, and permeability.
2) Undisturbed soil aggregate is used for determining aggregate stability.
3) Disturbed soil sample is used for determination of water content, chemical analysis, soil texture, particle density, high pF (pF 4.2), and bulk density (auger hole method).

In CSM BGBD project, the three types of soil samples are taken in each sampling point where the soil fauna groups obtaining their sample as well as at certain location in the windows areas. In sampling point level, the main purpose of the soil sampling is to characterize the sampling point from the viewpoint of physical and chemical properties. The soil samples are taken about 7 m from the center of monolith, where the soil fauna group are taken their sampling, as shown in Figure 1. In window level, the purpose of the soil sampling is to classify the soil based on FAO and USDA soil taxo-no-my. So, the soil samples are taken from the profiles.

5.1 Field Soil Sampling Methods

Point sampling level

The purpose of soil sampling in point level is to analyze several physical properties as well as soil chemical properties. The physical properties include bulk density, particle density, soil texture, distribution of pore (pF), soil color, shape of aggregate; while the chemical properties include pH, C-organic and N-total, and other chemical properties which are considered important to soil fauna.

In each sampling point, the soil samples are taken in three depths: 0"10 cm, 10"20 cm, and 20"30 cm. The types of soil samples are as follows:

1) Disturbed soil samples are taken using small spade from three sites around the sampling point (Figure 1), and mixed to get a composite.
2) Undisturbed aggregate soil samples are also taken from three locations, and mixed to get a a composite.
3) For bulk density, the auger hole methods is used. A small auger with 5 cm thick is inserted into the soil, and the soil in the auger is removed and put into plastic bag.

Profiles soil sampling

Soil profile is dug up to 1.5 m depth and each layer is sampled as follows:

1) Disturbed soil samples are obtained for chemical and certain soil physical analysis.
2) Undisturbed core samples (5 cm thick, 5 cm diameter) are prepared for pF determination and bulk density, with 3 replicates per sampling.
3) Morphology of the profiles are observed and identified.

5.2 Laboratory Methods of Soil Physical Properties

Soil processing

Soil samples are air dried under shade after breaking the clumps. Dried samples are hand pound and passed through a 2-mm screen. The core samples as well as aggregate samples are saturated before being analyzed.

Soil physical analyses

a. The determination of soil moisture content (gravimetric method)

1) Weigh a metal can and record this weight as the weight of metal can (A).
2) Place a soil sample of about 10 g in the metal can and weighted (B).
3) Place the sample in the oven, set the temperature at 105°C, and dry for 24 hours or over night.

4) Take the sample from the oven and put in the desiccators until cool enough.
5) Weigh the sample, and record this weight (C)

Calculation: $W\ \% = \frac{Mw - Mp}{Mp} \times 100$

Where W = soil moisture content (gravimetric)
Mw = weight of wet soil
Mp = weight of oven dry soil

In this case the soil moisture content is

$$W = \frac{(B - C)}{(C - A)} \times 100$$

To convert into volumetric based, the result is multiplied by bulk density:

$$\theta\ \% = w \times \frac{\rho b}{\rho w}$$

b. The determination of bulk density (auger hole methods)

1) Determine the diameter and the height of the inner cylinder, and calculate the volume (V).
2) Push the auger sampler to the ground until 5 cm depth.
3) Withdraw the sample from the ground, and make sure to retain the soil sample undisturbed in the inner cylinder. Use the knife or the spatula to trim the soil extending beyond the edges of the inner cylinder.
4) Remove the soil from the auger, and put into the plastic bag which has known the weight (A).
5) Bring the soil sample to the lab and weigh with the plastic bag (B).
6) Dry it to constant weight according to the procedure is given in *Measurement of soil moisture content*.

The bulk density could be calculated as follows:

$$\rho b = \frac{Mp}{Vt}$$

Where ρb = mass of dry oven soil = (B -A)/(1+W)
Vt = volume of auger
W = soil moisture content

c. The determination of bulk density (ring sample method)

1) Place the soil sample in the ring sampler in the oven, adjust the temperature to 105°C, and dry for 24 hours or over night.
2) Take the sample from the oven and place it in the desiccators until it becomes cool.
3) Weigh the sample, and record this weight (A).
4) Remove the soil from the ring sampler, and wash the sampler, dry it, and weigh (B).
5) Determine the diameter (d) and the height of sampler (h), and calculate the volume (V).

$$\rho b = \frac{Mp}{Vt} \quad \text{and} \quad Mp = A - B$$
$$V = 3.14 \times (d/2)^2 \times t$$

Where ñb = bulk density (g cm^{-3})
Mp = mass of oven dry soil
Vt = soil volume
d = diameter of ring sampler
t = height of ring sampler
A = weight of soil and ring sampler
B = weight of ring sampler

d. The determination of particle density

1) Weigh a picnometer with capacity 25 cm^3 (A).
2) Grind air dry soil (diameter < 2 mm) and put 10 g into picnometer, and weigh (B).
3) Put water into picnometer about 2/3 of the volume and shake it.
4) Place in the hotplate until the air is out.
5) Cool the picnometer, and fill with water until the meniscus, and weigh (C).

$$\rho b = \frac{Mp}{Vp}$$

Where Mp = Mw / (1 + w)
$Volume_{soil}$ = $Volume_{picnometer}$ - Volume water
$Volume_{picnometer}$ = 25 cm^3
Volume water = weight of water = (C - B)

e. The determination of soil texture - hydrometer methods

1) Weigh a sample 50 g of oven dry soil, and place it in the Erlenmeyer.
2) Add, approximately 50 ml of 5% Calgon solution and 200 ml distilled water, and let it stand for one night.
3) Remove it into beaker, and stir for about 5 minutes.
4) Remove to the graduate cylinder, and add distilled water to make exactly 1 liter.
5) Mix the suspension thoroughly with the plunger, and record the suspension's temperature (T_1).
6) Gently, place the hydrometer at the top of the suspension, and record the scale reading (at the upper edge of the meniscus surrounding the stem), after 40 seconds (H_1).
7) Remove the hydrometer and wipe it with dry soft towel.
8) Take hydrometer readings after 120 minutes (H_2) and record the temperature (T_2).

$$\% \text{ clay + silt} = \frac{\{(H_1 - B_1)\} + FK}{Mp} \times 100$$

$$\% \text{ clay} = \frac{\{(H_2 - B_2)\} + FK}{Mp} \times 100$$

Where FK = temperature correction factor is 0.36 (T°C - 20°C)
Mp = weight of oven dry soil used in analysis

Hence:
% sand = 100 - (% clay + silt)
% silt = 100 - (% clay + sand)

f. pF determination (low pressure- pF 1,2, and 2.54)

1) Weigh the saturated ring soil sample (A).
2) Put the saturated ring soil sample into the ceramic plate.
3) Transfer the plates along with the soil samples to the pressure chamber. Bolt it tight.
4) Turn on the compressor and adjust the pressure of the chamber to positive pressure at 10 cm (0.01 bars). This pressure is indicated by the air bubbles which are flowing out.
5) Let the extraction proceed for 48 hours.

6) Weigh the ring soil sample, and return it to the chamber, and adjust the pressure to 100 cm (0.1 bars).
7) Repeat the procedure 5 and 6, and this time with the pressure 0.3 bars.
8) Weigh the ring soil sample, and transfer quickly to oven for 24 hours at 105 °C.

Calculation :

- *pF determination (high pF – pF 4.2)*

1) Acquire a 100 g of air-dried soil. Grind the soil and pass through the 2 mm sieve to remove all soil aggregates that is less than 2 mm diameter. Repeat this step for each soil sample.
2) Place the soil sampling rings onto the plates, and fill the rings with soil. Mark the sampling rings to distinguish between different soil samples.
3) Place the plates and the soil samples in trays. Wet the soil samples, and let then stand in a thin layer of water for approximately 16 hours or overnight.
4) Transfer the plates along with the soil samples to the pressure chamber. Bolt it tight.
5) Apply a positive pressure to 15 bars.
6) Let the extraction proceed for 48 hours or when water outflow ceased. Some soil reaches equilibrium in an overnight or 18-20 hours.
7) Determine the soil moisture content on weight and/or volumetric basis. (See *Measurement of soil moisture content by gravimetric method).*

- *pF determination-vaporation methods*

1) Weigh 5 g of soil, based on the calculation of absolute dry weight, in a weighing can.
2) Add water to make the soil wet enough.
3) Add some salt into the bottom part of the desiccators.
4) Put a weighing can filled with soil sample inside.
5) Close the desiccators. If the desiccators have a hole for air sucking, use a vacuum pump so that the vapor pressure balance could be achieved quickly.
6) Let the sample for 7 days before reweigh the soil with its weighing can.
7) Return the soil sample with its weighing can, and reweigh after 4 days.
8) Repeat the procedure several times until we get constant soil weight.
9) Calculate the soil moisture content after reaching constant weight.

According to Soekodarmodjo *et al.* (1984) as cited by Afandi *et al.* (2005), the pF value can be integrated from:

$$pF = 6{,}5 + \log (2 - \log RH)$$

The following table shows types of salt with their pF values.

Table 8. Salt and its pF value

Salts	Relative humidity (%)	pF values
$(NH_4)_2CrO_4.H_2O$	98.8	4.20
$CaSO_4$	97.8	4.49
K_2SO_4	97.1	4.60
$NH_4H_2PO_4$	93.0	5.00
K_2CrO_4	88.0	5.20
NH_4Cl	79.3	5.51
NaCl	75.8	5.60
$Mg(NO_3)_2$	52.0	5.96
$KC_2H_3O_2$	19.9	6.36

g. The determination of soil structure

Soil structure can be evaluated by taking a sample of undisturbed soil (either from the pit or from the shovel or auger) and holding it in your hand. Then, look closely at the soil in your hand and examine its structure. As a guide, here are possible choices of soil structure.

- **Granular:** Resembles cookie crumbs and is usually less than 0.5 cm in diameter. Commonly found in surface horizons where roots have been growing.
- **Blocky:** Irregular blocks that are usually 1.5" 5.0 cm in diameter.
- **Prismatic**: Vertical columns of soil that might be a number of cm long. Usually found in lower horizons.
- **Columnar**: Vertical columns of soil that have a salt "cap" at the top. Found in soils of arid climates.
- **Platy**: Thin, flat plates of soil that lie horizontally. Usually found in compacted soil.
- **Single Grained**: Soil is broken into individual particles that do not stick together. Always accompanies a loose consistence. Commonly found in sandy soils.
- **Massive**: Soil has no visible structure, is hard to break apart and appears in very large clods.

h. The determination of soil color

1) Take a ped of soil from each horizon and note on the data sheet whether it is moist, dry or wet. If it is dry, moisten it slightly with water from your water bottle.
2) Break the ped.
3) Using "Munsell Soil Color Chart", examine the color of the ped.

Note: *Sometimes, a soil sample may have more than one color. Record a maximum of two colors if necessary, and indicate (1) the main (dominant color) and (2) the other (sub-dominant color).*

5.3 Laboratory Methods of Soil Chemical Properties

Chemical analyses of soils are done for many purposes. This section is concerned with the use of these analyses to determine the available nutrient status of soils. The usefulness of these analyses is determined by our ability to translate them into intelligent management recommendations. These recommendations depend not only on accurate sampling and analysis but also on interpretations based on sound research and judgments. Presented here are brief sampling instructions and selected analytical methods.

Sampling

A good sample is the first requirement for a reliable soil test. The proper method of collecting and handling samples is determined by (1) the use to be made of the analyses, (2) the pattern and ease of recognition of soil variability, and (3) previous and proposed management practices.

Soils vary in their vertical and horizontal dimensions. These variations result from natural (soil development) and man-caused (fertilization, leveling, irrigation) forces and occur on both micro and macro scales. Microvariation (that within the rooting area of a plant) results from such factors as the localized effects of plants, urine spots, rodent activity, or fertilization and contributes about one-half of the total variance of the sample. Thus, it is best averaged by compositing several sub-samples into a single sample. The determination of soil chemical properties requires disturbed soil sample from three depths of 0"10, 10"20, and 20"30 cm (see the beginning of this chapter on pages 42"43).

Analytical methods

Laboratory assays of soil fertility most commonly involve determination of the concentrations of nutrient elements in a soil extract or equilibrated solution. The choice of extractant is critical since different extractants remove different amounts of nutrients from soils and thus require different interpretive calibrations. Some procedures give values better related to crop responses on some soils than do others.

Nonetheless, the "standard" procedure for soil chemical analyses may vary from one laboratory to another. Thus, we hold back to provide the details for each method since most researchers practically send soil samples to the nearest laboratory and let the laboratory for doing so. Comprehensive methods of soil chemical analyses are described elsewhere see Brown *et al.* (1986), Chapman (1965), Gaines and Mitchell (1979), Kjeldahl (1883), Jones (1991), Page *et al.* (1982), Thomas (1982), Walkley and Black (1934), and many more.

Chemical properties of soil are numerous and measuring all of them require substantial efforts. The choice of what to be measured should depend on the purpose of collected data and other common constrains such as money, time, and available laboratory facility. The following are major soil chemical properties, which determined for CSM-BGBD study in Indonesia, and the method to measure them.

1) Soil pH (H_2O and KCl) is determined by a 1:5 extraction method and measured with a glass electrode pH meter previously calibrated with standard solution.
2) The determination of C-organic is done by using Walkley-Black methods.
3) Total soil nitrogen is measured by using semi-micro Kjeldahl method.
4) Cation exchange capacity (CEC) is determined by extracting soil sample with 0.1 N NH_4Oac (amonium acetate) at pH 7.0 and measured with an auto analyser apparatus.
5) Exchangeable cations (K, Na, Ca, and Mg) are extracted by 0.1 N NH_4Oac (amonium acetate) at pH 7.0 and measured with a flame photometer and atomic absorption spectrophotometry (AAS).
6) Base saturation (BS) refers to the number of basic cations that are held on the soil exchange (CEC sites) in comparison to the total number of sites, i.e. the quantity of Ca^{2+}, Mg^{2+}, and K^{+}, in meg/100 g, divided by the measured CEC.

7) Exchangeable acidity of aluminum (Al) and hydrogen (H) are extracted by 1 N KCN by titration method.
8) Free Fe and Al are determined by extraction with citrate-dithionite (CD) and measured with AAS.
9) The determination of potential P and K is done by extraction with 25% HCl.
10) Available P is determined by using Bray-I method.

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