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# The role of soil fauna in litter decomposition in primary forests, secondary forests and a polyculture plantation in Amazonia - methodological considerations

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## ABSTRACT

The project SHIFT ENV 52 undertakes a comparative study of litter quantity and quality, decomposition rates, and the abundance, biomass, and respiration of soil-inhabiting microbes, arthropods and oligochaetes in a polyculture forestry plantation and in plots of nearby secondary and primary forest. The aim is to evaluate the specific contribution of the soil microflora and of the different functional soil fauna groups to the decomposition of organic matter and the resulting nutrient supply to the plants. Several methods for the study of the soil fauna and microflora have been adapted to neotropical conditions on the base of preliminary tests. This article gives an overview.

## RESUMO

O projeto SHIFT ENV 52 faz um estudo comparativo de quantidade e qualidade de serrapilheira (liteira), taxas de decomposição, e de abundância, biomassa e respiração de microorganismos, artrópodos e oligochaeta do solo numa plantação de cultivo mixto, e em áreas de floresta primária secundária e primária adjacentes. O objetivo é de avaliar a contribuição específica da microflora do solo e dos diferentes grupos funcionais da fauna de solo à decomposição da matéria orgânica e o resultante suprimento de nutrientes para as plantas. Vários métodos para o estudo da fauna e microflora foram adaptados na base de testes preliminares às condições neotropicais. Este artigo dá um resumo.

## ZUSAMMENFASSUNG

Das Projekt SHIFT ENV 52 unternimmt eine vergleichende Untersuchung von Streumenge und -qualität, Abbauraten, und der Dichte, Biomasse, und Atmung von bodenlebenden Mikroorganismen, Arthropoden und Oligochaeten in einer Mischkultur-Plantage und in Flächen von benachbartem Sekundär- und Primärwald. Das Ziel ist die Evaluierung des spezifischen Beitrags der Mikroflora und verschiedener funktioneller Bodenfaunagruppen zum Abbau der organischen Substanz und die resultierende Nährstoffversorgung der Pflanzen. Verschiedene Methoden für die Untersuchung der Fauna und Mikroflora wurden auf Grundlage von Vorversuchen an neotropische Konditionen angepaßt. Dieser Artikel gibt eine Übersicht.

## INTRODUCTION

Considerable areas of the rain forests of Amazonia (Brazil) are characterized by nutrient poor soils, which make sustainable agri- and silviculture difficult. To develop an ecologically, socially and economically viable system of agriculture better suited to the conditions of Amazonia, several projects are carried out at the Brazilian Agroforestry Research Facility Embrapa (Empresa Brasileira de Pesquisa Agropecuária na Amazônia Ocidental) in Manaus (Amazonas State, Brazil) within the German-Brazilian Scientific Cooperation Program "Studies on Human Impact on Floodplains and Forests in the Tropics " (SHIFT) (projects ENV 23, 42, 45). The principal aim of these projects is to develop methods for sustainable land use in Amazonian rain forests, using an experiment on recultivation of a fallow rubber plantation with mixed plantations of annual and perennial plants (polyculture systems). In 1997, a new project on soil fauna and litter decomposition (ENV 52) was established, closely related to the existing SHIFT projects in Manaus. The present paper is a general description of this project. In the project ENV 52, litter quantity and quality, decomposition rates, and the abundance, biomass, and respiration of soil-inhabiting microbes, arthropods (especially collembolans, mites, ants, spiders and termites) and earthworms are studied comparatively in one of several different polyculture systems (a forestry plantation consisting of 4 tree species) and in plots of nearby secondary and primary forest. In intact forests the soil fauna and microorganisms are abundant and extremely diverse, building a complex network of interacting processes. In secondary forests and plantations abundance and diversity of soil organisms may be altered, which possibly interferes in the nutrient cycling.

The aim of this study is to evaluate the specific contribution of the different functional soil fauna groups and of the soil microflora to the decomposition of organic matter and the resulting nutrient supply to the plants. Our basic hypothesis is that a functional soil fauna is of extreme importance for the maintenance of "healthy" nutrient cycles in the systems, and that biotic and abiotic factors of the sites can be managed in order to optimize the cycling of nutrients. In view of the high variability in the distribution of the fauna in tropical soils, only an exhaustive and very time-consuming sampling scheme will allow to address these questions and to provide a model of the underlying processes which will be applicable in similar situations. One aim, however, is to approach the establishment of short-term methods of bioindication of the "operative health" of the decay processes which allow less labor-intensive though significant sampling in future studies in the area of sustainable development.

Therefore, the central questions of our project are:

- Which species or functional groups belong to the decomposer communities of the primary and secondary forest and a polyculture plantation ?
- How do these communities, litterfall, litter standing crop, microbial biomass and nutrient conditions of these sites differ ?
- What is the quantitative role of different decomposer groups in the processes of decomposition and nutrient supply ?
- How can biomass of different soil fauna groups and decomposition rates be integrated into a model and used in predictions of sustainable forestry ?
- Which methods (e.g. bait lamina, microcontainers, litter bags) can be used as for a less labor-intensive assessment of the function of the biocenosis ?

Since the project ENV52 is still running (sampling started in July 1997 and will be continued at least until early summer 1999), this contribution focuses mainly on methodological considerations. For many methods used as pedobiological standards in studies

in temperate regions, adjustments had to be made for the tropical conditions of the site. The first results using these modified methods (not presented here) point to a considerable importance of the soil fauna in enhancing the litter decomposition in primary forests. However, these conclusions will have to be confirmed by future collections.

## MATERIAL AND METHODS

### Site description

The study area is located at km 29 on the road Manaus-Itacoatiara close to the agroforestral research station Embrapa Amazônia Ocidental in the state of Amazonas (Brazil). The main area, established in 1992 on an abandoned former plantation of rubber trees, consists of 90 experimental polyculture plots of 32 x 48 m each (in total 18.9 ha). All samplings of the project ENV52 are done simultaneously on three plots (Figure 1):

- the polyculture system 4, a silvicultural plantation of four different tree species (*Hevea* spp. (Euphorbiaceae), *Schizolobium amazonicum* (Caesalpinaceae), *Swietenia macrophylla* (Meliaceae) and *Carapa guianensis* (Meliaceae), planted in 1992, with secondary vegetation admitted between the tree rows;
- a secondary forest, growing since 1984 immediately nearby on the same ground as the whole experimental plantation; it is dominated by three *Vismia* sp. species;
- a primary forest (terra firme), in close neighborhood of the experimental plantation.

Today, six years after initiating the plantation the silvicultural system is still in an early stage of development and the secondary vegetation between the tree rows clearly dominates the site (regarding plant cover and especially litter production). The litter layer in the rows (in fact, paths) below the planted trees is strongly disturbed. Therefore, the system could also be regarded as a young secondary forest rather than a tree plantation. We may say that, the soil fauna and microflora of a young secondary forest is compared with an elder secondary forest and a primary forest.

In each of the secondary (SEC) and primary forest (FLO) sites, one plot of 40 x 40 m was marked, whereas in the polyculture two subplots (each 32 x 48 meters) were defined (POA, POC). The main soil properties are practically the same at all plots (cf. Table 1 in Römbke et al. - this issue). Long-term measuring devices were exposed in the study sites (litter-sampler, temperature and humidity-loggers) in order to study litter input and climatic parameters.

Monthly rainfall at the study site was much below the 10-year mean in the months May, June, July, September and October 1997. Monthly air temperature means were higher than the 10-year means in all months since August 1996 and very high in September and October 1997. Relative air humidity was very low in July, September and October 1997, and monthly evaporation means very high from June to October 1997, when compared with the long-term means. In 1997, the annual mean temperature was 27.9 °C and the precipitation was 2582 mm. For the whole region of central Amazonia, effects of a suspected El Niño were discussed in 1997. Therefore, the data collected in 1997 may well represent an untypical, extraordinarily dry year.

## Sampling and extraction methods

The sampling program is performed simultaneously in the three forest plots described above. Two series of litter bags are used for the investigation of the decomposition processes, one started in the middle of the dry season in October 1997 and the other started in the rainy season (April 1998). Litter production and litter stocks are collected continuously during the whole project period. Sampling of meso- and macrofauna and samples for microbial respiration measures are taken synchronously in three month intervals, starting prior to the first litter bag series and ceasing together with the second series. Chemical analyses (C/N-ratio, macro- and micronutrients, exchangeable cations, humic substances) of soil, plant residues and soil animal products allow to estimate the contribution of each faunal group to the turnover of selected elements. Four sampling events have been carried out on schedule since July 1997. The last sampling event of a total of eight is planned for early summer 1999.

**Soil organisms.** Since standardized soil biological methods for neotropical rain forests are not available, methods developed for temperate regions had to be adapted on the base of preliminary tests. The following modified methods for the study of soil organisms (especially all soil macrofauna of >2mm body diameter and either saprophagous or with predatory habits) are used:

- Extraction of the soil macrofauna from large soil-cores (diameter: 21 cm) by means of a modified Kempson-apparatus for the determination of species composition, abundance and biomass (e.g. Adis 1987);
- Additional stratified and bait sampling for termites and ants because social insects are not adequately sampled with the classical soil core extraction;
- Manual sampling for very large macrofauna from the litter layer in areas of 4 m<sup>2</sup>;
- Formol-extraction of mainly glossoscolecoid earthworms in areas of 4 m<sup>2</sup>;
- Soil samples taken with split-core samplers (diameter 6.4 cm) for the dry extraction of Mesoarthropoda (mainly mites and collembolans) using a Berlese funnel and of enchytraeids by means of a wet extraction method;
- Experimental enhancement and exclusion of predators for the assessment of the impact of predators on saprophagous soil fauna (macro- and mesofauna);
- Measurements of the respiration rates of selected soil animals like termites (using an Infrared-Gas-Analyzer - IRGA) together with population estimates will allow to assess turnover rates of the different soil fauna groups;
- Measurement of microbial respiration and biomass of the litter layer and the uppermost 5 cm of the soil using an IRGA.

The determination of the species level or of functional groups has been performed so far only to a limited extent due to the poor knowledge on soil animal taxonomy or biology in tropical rain forests. However, it is planned to determine most organisms to the species level.

Three examples of modifications should briefly be mentioned:

The sampling program for large Oligochaeta was defined on the basis of numerous pre-trials. The method used now, which consists in expelling large (mainly glossoscolecoid) earthworms from a 4-m<sup>2</sup>-area with 80 liters of a 0.25 % formol solution applied over 30 minutes, is superior to hand sorting which is generally recommended in the literature. For the first time it proved to be successful in the tropics.

Termite baits for comparative studies of the termite populations of the sites, and which also are used for the supply of soil termites for respiration measurements, were successfully established in the second part of the year. An overall acceptance of 40 % was recently recorded (in contrast to older findings in which only 10 % of the baits were accepted).

In May 1998, a sampling program for the determination of diversity and biomass of termites has been started, which together with respiration measurements and studies of feeding guilds of termites will allow to assess the impact of this faunal group in decomposition processes. The sampling program is based on the standard protocol suggested by Eggleton and Bignell (1995), however, we have been modifying the procedures to reduce the total processing time (given as 19 man-months for 1 hectare), e.g. by using a different shape of the sample area and substituting hand-sorting with Kempson extraction.

**Microbial measurements.** Every three month, 20 soil samples (top soil 0-5 cm and 5-15 cm) and mixed litter samples from the soil surface are sampled on each plot with a soil corer (6.5 cm diameter). Soil samples are sieved (< 4 mm) and stored at 10° C until used. The amount of microbial biomass in the soil and in the litter is estimated by applying the Substrate-Induced Respiration (SIR) method (Anderson and Domsch 1978). Metabolic activity is assessed by measuring the basal respiration (BR) of the soil and of the litter. If necessary, soil and litter were adjusted to the optimal moisture (ca. 40 % WHC) and pre-incubated before measuring SIR. All measurements are done in a continuous flow-through system connected with an Infra-Red Gas Analyzer (IRGA) under controlled temperature conditions.

**Functional measurements.** Litter production is measured weekly over 24 weeks with 20 litter samplers (surface area: 0.25 m<sup>2</sup>) on all three plots. Additionally, litter stocks are monthly collected over 5 months using 20 samples (large soil-cores of 21 cm diameter) on each plot. Litter stock is also determined by monthly sampling of the litter from a 4 m<sup>2</sup> area.

Before starting the litterbag experiment, it had to be determined which mesh size is best suited for this purpose in tropical areas (Anderson et al. 1983). In a pre-study in 1996 mesh sizes of 0.02, 0.25, 0.5 and 10 mm were tested. As the incidence of macrofauna was sharply reduced from the 0.5 mm bags to the 0.25 mm bags, it was concluded that the use of the mesh sizes 0.02, 0.25 and 10 mm is most suitable to distinguish between the contributions of microflora, meso- and macrofauna in the tropics (Beck et al. This issue). In fact, the same mesh sizes were proposed during a meeting of the German Working Group on Mesofauna (Ahrens et al. 1989), based on the experiences of seven working groups, covering mainly forest and agricultural sites in temperate regions. Therefore, in October 1997, the first litterbag series with 1008 litterbags containing air-dried *Vismia*-litter to evaluate site-specific decomposition rates was distributed in the study sites (Figure 2). *Vismia* was chosen because it represents a litter type that is present in all three forest types. The retrieval of the bags from randomised positions started 4 weeks later and has gone on since then. However, the litter bag program initially designed to last 6 months was extended to 1 year in view of the low decomposition rates of the litter of *Vismia guianensis* used as a standard in this study.

Together with the litterbags a series of minicontainer bars was distributed in the field as a second method to measure decomposition rates (Eisenbeis 1994). For the first time, the minicontainer system is tested as an alternative to the litterbags in the tropics (Figure 3). Since The twelve small individual containers (diameter: 2 cm), forming one minicontainer bar, and also filled with *Vismia*-litter, are handled as independent units. Due to this high number of replicates and the small amount of litter in each of the individual containers, it is expected that

this method can be used as a quick and statistically reliable (screening) method for the determination of decomposition rates.

The latest approach for testing functional endpoints is the bait-lamina-test (Von Törne 1990a,b). Assessment endpoint is the feeding activity of a variety of soil animals (e.g. Collembola, Enchytraeidae) whereas the activity of micro-organisms is not detectable (Figure 4). The absolute amount of fed material as well as the vertical distribution of the feeding activity are recorded and statistically evaluated. Since environmental conditions like climate or soil moisture can influence strongly the results, the method should only be applied for comparing the biological activity between closely related plots like in our project measured simultaneously.

Its use on the three plots was tested in July; its regular use started in December. The adequate exposure time of the bait lamina turned out to be much shorter than in extratropical studies where up to 28 days are often used (Federschmidt and Römbke 1994). In July, 80 % of all holes were open within 19 days. Four days already allowed an evaluation of the frequency of empty holes caused by faunal feeding in December 1997. The feeding activity was higher in the plantation sites than in primary and secondary forest sites, both during the preliminary test in July and the regular exposition in December. This result probably reflects the problems encountered with the use of such unspecified „activity“ measures. Considerable calibration with standard faunal assessments is needed before bait lamina experiments will yield reliable data.

## DISCUSSION

Up to now, no standard set of methods for soil ecological investigations in tropical rain forests exists. The only text coming next to such a standard is the well-known "Handbook of Methods of Tropical Soil Biology and Fertility", first published in 1989, and later revised (Anderson and Ingram 1993). This book focuses mainly on soil fertility and gives very detailed descriptions of soil chemical and physical methods. Unfortunately, the handbook is far less complete in the field of soil biology. Many methodological suggestions for the investigation of various animal groups as well as for the adaptation of functional measurements like organic matter decomposition are scattered in the literature. Even the most comprehensive manual for the study of soil biology is only available in German and does cover mainly temperate regions (Dunger and Fiedler 1997).

Therefore, the first outcome of the project ENV52 is a compilation of soil biological methods for the use in tropical rain forests. The established sampling methods together with work plans and detailed step-by-step instructions for many procedures have been compiled into a „handbook“, accessible via internet from our homepage (since March 1998): <http://www.cenargen.embrapa.br/~mgarcia/shift>.

The application of the results of this study to other, similar areas, and the transfer of the conclusions for practical purposes (improvement of sustainability of land-use techniques in the humid tropics) is supposed to be facilitated by the planned parallel characterization of the study sites. A comprehensive evaluation of the data in co operation with other SHIFT projects jointly studying the EMBRAPA site will be possible at the end of the investigations.

The investigation will produce

- basic data about dynamics of decomposition processes in a tropical forest and the specific contribution of the different organisms and functional groups (microflora, mesofauna, macrofauna)
- comparable data for the polyculture plantation and the secondary forest both stocking on a fallow rubber plantation
- data on leaf and wood litter "standing crop" and production in the stands of the three vegetation types.

The integration of these data together with biotic and abiotic data available for study areas (e.g. vegetation cover, secondary growth biomass, microclimate) should allow to assess the nutrient supply of the polyculture system and the role of the soil biota in this process.

By testing and comparing different methods to study decomposition the project it is hoped to develop standards and rapid applicable procedures to measure decomposition rates and the role of the pedofauna and microflora in the field for the practical purpose of assessment of the sustainability of management measures. The resulting model of the underlying biotic and abiotic processes should in turn permit

- a) to establish the methods for rapid assessment of soil biota function, and
- b) the management of the factors controlling soil biota in order to optimize nutrient cycling.

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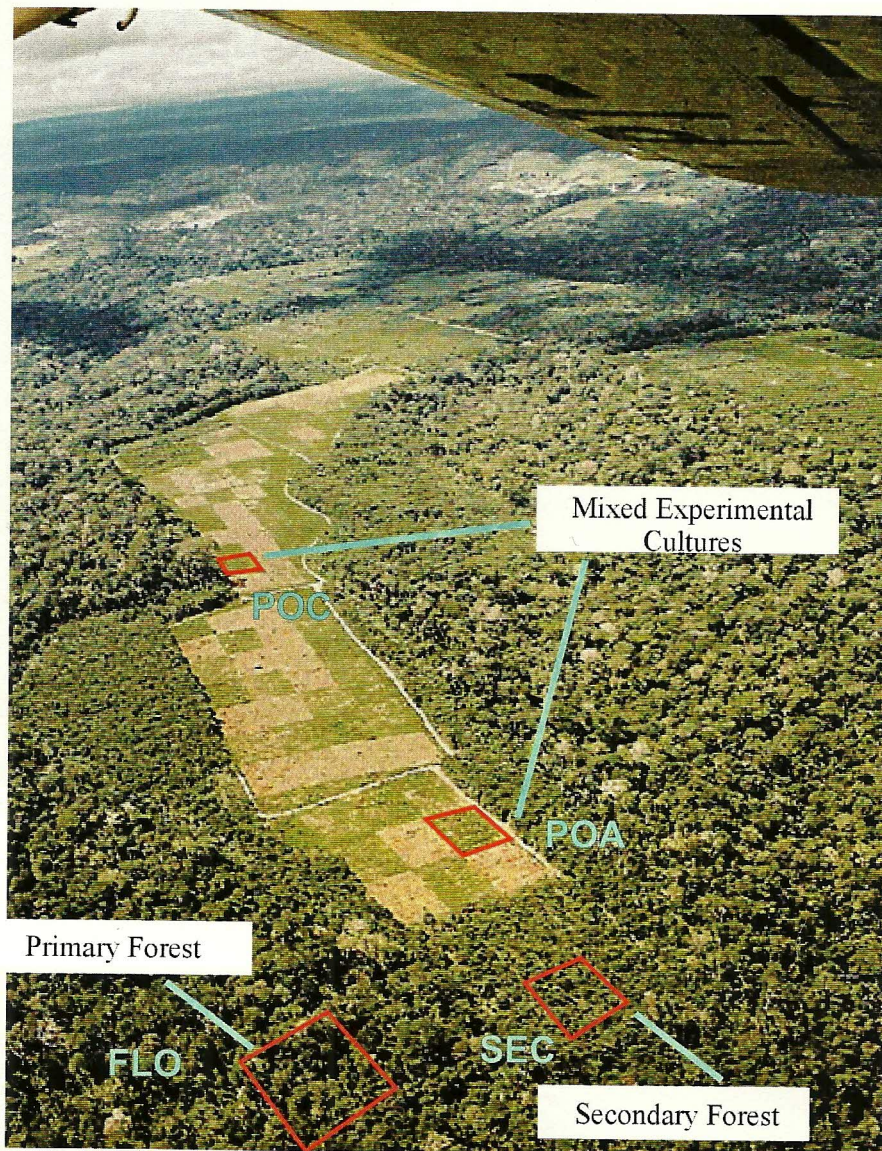
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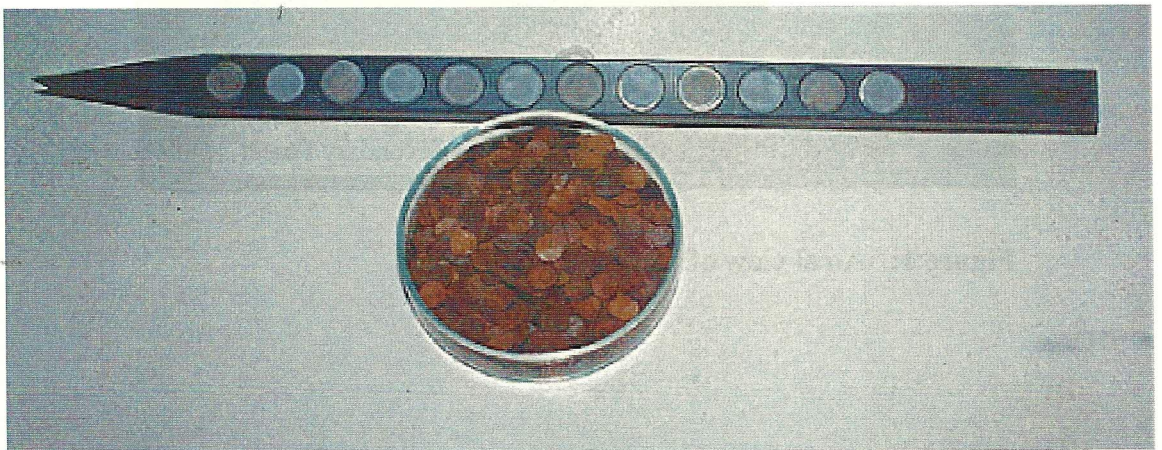
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**Figure 1:** Areal view of the study area.



**Figure 2:** Litter bags and minicontainer exposed in the field.



**Figure 3:** Detailed view of a minicontainer bar.