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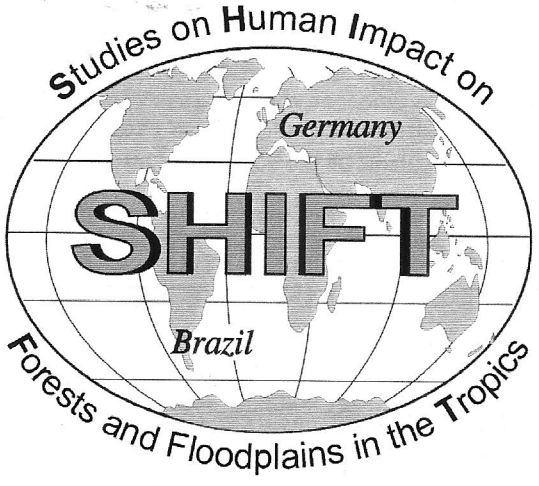

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Soil fauna and litter decomposition in primary and secondary forests and a polyculture system in Amazonia - study design and methodology

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ABSTRACT

A comparative study of litter quantity and quality, decomposition rates, and the abundance, biomass, and respiration of soil-inhabiting microbes, arthropods and oligochaetes has been started in 1997. The three study sites are a primary forest, a 13-year old secondary forest and a polyculture plantation system consisting of four commercially used tree species planted in rows between adventitious vegetation. 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. A general description of the study design of the project is given and some methodological aspects are discussed. Methods that are standard in soil biological studies in temperate regions had to be adapted to tropical conditions. For studies of litter decomposition rates using litter bags, we suggest standard mesh sizes to be used for an ecologically relevant separation of meso- and macrofauna.

RESUMO

Um estudo comparativo de quantidade e qualidade de liteira, taxas de decomposição, e de abundância, biomassa, e respiração de microorganismos, artrópodes e oligochaeta de solo foi iniciado em 1997. Três locais são estudados, uma floresta primária, uma floresta secundária de 13 anos de idade e um sistema de plantação de policultivo que consiste em quatro espécies de árvores comercialmente usadas e plantadas em filas entre vegetação adventícia. O objetivo é avaliar a contribuição específica da microflora de solo e dos diferentes grupos funcionais de fauna de solo para a decomposição de matéria orgânica e a resultante provisão de nutrientes para as plantas. Uma descrição geral do desenho de estudo do projeto é dada e alguns aspectos metodológicos são discutidos. Métodos-padrão em estudos de biologia de solo em regiões temperadas tiveram que ser adaptados a condições tropicais. Para estudos da taxa de decomposição da liteira com litterbags (sacos de liteira), sugerimos, em base de experimentos preliminares feitos em 1996, que sejam usados tamanhos-padrão de malha para uma separação ecologicamente pertinente de meso- e macrofauna.

ZUSAMMENFASSUNG

Eine vergleichende Untersuchung von Menge, Qualität und Zersetzungsrate der Bodenstreu sowie Dichte, Biomasse und Atmung der bodenlebenden Mikroorganismen, Arthropoden und Oligochaeten wurde 1997 auf drei Untersuchungsflächen begonnen. Es handelt sich um einen

Primärwald, einen 13-jährigen Sekundärwald und eine Mischkultur-Plantage. Letztere besteht aus vier kommerziell genutzten Baumarten, in Reihen gepflanzt auf einer Sekundärwaldrodung, zwischen gemeinsam mit aufwachsender Sekundärvegetation. Ziel der Untersuchung ist, den spezifischen Beitrag der Bodenmikroflora und der verschiedenen funktionellen Bodenfaunagruppen zur Zersetzung der organischen Substanz und zur daraus resultierenden Nährstoffversorgung der Pflanzen abzuschätzen. Das Untersuchungsdesign des Projekts wird beschrieben, und einige methodologische Aspekte werden diskutiert. Methoden, die in bodenbiologischen Studien in gemäßigten Breiten Standard sind, mußten an tropische Verhältnisse angepaßt werden. Für Studien der Streuzersetzungsraten mit Hilfe von Streubeuteln schlagen wir, auf der Basis einer 1996 durchgeführten Voruntersuchung, die Verwendung von Standard-Maschenweiten für eine ökologisch relevante Trennung von Meso- und Makrofauna vor.

INTRODUCTION

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 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, our project on soil fauna and litter decomposition (ENV 52) was established, closely related to the existing SHIFT projects in Manaus.

The underlying assumptions are that soil fauna and microflora communities are extremely important for the maintenance of functional nutrient cycles in the systems (Beck et al. 1997; Lavelle et al. 1993, 1994) and that biotic and abiotic factors may be manipulated to optimize the composition of the soil biota, to guarantee the efficient recycling of nutrients and their conservation in the system (Pashanasi and Lavelle 1989, Tian et al. 1993). Parameters like the quantity and quality of the litter produced in the systems, the decomposition rates, and the abundance, biomass and respiration of microorganisms and soil animals are simultaneously and comparatively studied in primary and secondary forest and one of the polyculture systems.

Our aim is to obtain data on the specific contribution of the microflora and soil fauna to the decomposition of the organic matter, and on the importance of these processes for the nutrient supply to the plants. In particular, we will focus on studies of the interaction between microflora and soil fauna, an aspect that, up to now, has been poorly studied in the tropics.
Field work started in July 1997 and will go on until April 1999.

BASIC STUDY DESIGN

We study three different systems, located in sites within and close to the experimental 17 ha-area of the EMBRAPA Amazônia Ocidental at km 24 on the road Manaus-Itacoatiara in Central Amazonia:

- the polyculture system 4, a forestry plantation of four different tree species, planted in 1992, with secondary vegetation admitted between the tree rows;

- a secondary forest, growing since 1984 on the same ground as the whole experimental plantation, which formerly was an abandoned rubber tree monoculture;
- a primary forest, in close neighborhood of the experimental plantation.

Within the experimental plantation, the polyculture system 4 was laid out in 5 replications included in the block design (block A-E) of the whole experiment. However, preliminary studies (Preisinger et al. 1994) have shown that two blocks (D and E) strongly differ from the others in important characteristics like vegetation cover, previous intensity of land use and water drainage.

The number of samples that can be taken at one time in our project is limited by the number of places in the extraction apparatus (Berlese and Kempson extractors) and principally by the time needed to sort and determine the collected animals and to process the withcoming substrates.

In view of the fact that few comprehensive studies of soil fauna in the tropics exist and that the high diversity and variability of tropical areas requires a large number of samples to be taken at each sampling event, we decided to collect higher sample numbers within the studied systems instead of replicates of the systems. Also, several subsites in a highly diverse primary rain forest would hardly represent realistic replicates of a "treatment" and replicates of the "polyculture tree plantation" were not available in sufficient number (see above). Generalization of conclusions will consequently not be based on a statistical evaluation of the effect of the treatment (expected to be act through a variety of factors), but rather on quantitative modeling of all soil fauna parameters of the three systems based on reliable means (high sample numbers) and good functional knowledge of the links between the model elements.

The single plantation sites are rather limited in size (32 x 48 meters) and the litter layer in the rows (in fact, paths) below the planted trees is strongly disturbed. Consequently the area necessary to sample the parameters of our program during a one year period can only be obtained by using two sites of the polyculture plantation (system 4), the one in block A and the one in block C of the experimental site. In the elder secondary forest and the primary forest we marked areas of 40 x 40 m as study sites.

At the time of the study, six years after the plantation was established the forestry system is still in an early stage of development and the secondary vegetation between the tree rows strongly dominates the site regarding plant cover and especially litter production. Consequently, our study system should be regarded as a young secondary forest rather than a tree plantation. Therefore, we prefer to say that we are investigating soil fauna, microflora and nutrient cycling in a young secondary forest (6 years) in comparison with an elder secondary forest (14 years) and a primary forest. The early state of such a "plantation" certainly determines the nutrient status of future states of the tree plantation, when the planted trees become higher, produce more litter and begin to dominate the area.

In addition to the basic sampling program important factors influencing the systems are being studied experimentally:

1. the effect of experimental increase of litter quantity in areas of 4 m^2 on microbial biomass, and nutrient (C, N, K, Ca, Mg, P) quantity;
2. the effect of increased and decreased number of arthropod predators (spiders) on macro- and mesofauna density and on litter decomposition rates.

These experimental studies test hypotheses about functional responses and will enable to link the parameters measured in the basic sampling program.

METHODOLOGY

The basic sampling program consisting of three-monthly sampling of fauna and microflora during 2 years was started in July 1997. Macrofauna is sampled by large soil core samples (21 cm diameter) from 20 randomized points per site, each separated in a litter and a soil portion (5 cm depth) and extracted in a Berlese apparatus. Additionally the litter of two quadrates of 4 m^2 each is hand sorted for larger macrofauna, that is insufficiently sampled with the soil cores.

Mesofauna is sampled by small soil core samples (6 cm diameter) from 20 randomized points per site, each separated in a litter and a soil portion (5 cm depth) and extracted in a modified Kempson apparatus (Adis 1987). At the same randomized points samples of the same size are taken for wet extraction of enchytraeids and for microbial biomass determination. The microbial biomass is determined with respiration measurements in an Infrared-Gas-Analyzer (IRGA).

Termite baits for comparative studies of the termite populations of the sites, which are also used for the supply of respiration measurements with soil termites, were successfully established in the second half of 1997. An overall acceptance of the baits of 40 % was recently recorded, in contrast to older findings in which only 10 % of the baits were accepted.

For many methods that are used as pedobiological standards in studies in temperate regions however, adjustments had to be made for the tropical conditions of the site. One example is that the adequate exposure time of bait lamina, which measure general feeding activities of soil fauna (Törne 1990a, b), turned out to be much shorter than in extratropical studies where 14 days are generally used.

The sampling program for large Oligochaeta was defined on the basis of numerous pre-trials. The established method consists in expelling large earthworms from a 4 m^2-area with 80 liters of a 0.25 % formol solution applied over 30 minutes. This is superior to hand sorting, which is generally recommended in the literature (Fragoso and Lavelle 1992, Anderson and Alexander 1993).

The two litter bag series initially designed to last six months were extended to one year in view of the low decomposition rates of the litter of *Vismia guianensis* (Guttiferae), used as standard litter in this study. *Vismia* was chosen because it is a dominant pioneer tree in many secondary forests in Amazonia; its litter is present in all sites under study.

In order to determine which mesh size is best suited for the purpose to discriminate between macro- and mesofauna, we performed a litter bag study in 1996 using the following mesh sizes: 0.02, 0.25, 0.5 and 10 mm. Ten litter bags of each mesh size were exposed in the field. They were made out of polyester gauze, had a size of 25 X 30 cm and were each filled with 10 g of air-dried leaves of *Vismia guianensis*. They were randomly distributed on the soil surface in the primary forest. During the period of exposure (one month) the average temperature was 24.9 °C (Max. 26.3 °C, Min. 23.2 °C) and the total precipitation was 195.6 mm (data from an automatic field station). The remaining litter was transported to the laboratory where the fauna was extracted using the Kempson apparatus.

RESULTS

Large numbers of mesofauna animals were found in the bags of the three larger mesh sizes, whereas almost none appeared, as expected, in the 0.02 mm bags. Exceptions (Table 1) can be explained assuming that eggs of Acari and Collembola were laid through the meshes of some bags. In individual cases, bags seem to have been damaged by roots or animal attack; thus allowing larger animals to penetrate than mesh size would permit. However, differences between the smallest and the other mesh sizes are highly significant for Acari, Collembola and the whole mesofauna (Mann Whitney U test: $p>0.001$), showing that access of even very small animals is effectively hindered.

Animals of the macrofauna groups were found only in small numbers in the 0.25 mm bags, but appeared much more abundant in the 0.5 mm bags (Table 1, differences are significant at the 0.1 % level for isopods, pseudoscorpions and spiders and the sum of all macrofauna groups, significant at 1 % level for diplopods). Both Acari and Collembola were found in higher numbers in the bags with 0.25 mm mesh than in the 0.5 mm bags (significant at 5 % level). This might be due to lacking competition with larger animals (macrofauna) or a protection effect of the 0.25 mm bags, which apparently did not allow access of the predator groups Araneae and Pseudoscorpiones. The 0.5 mm bags showed no significant differences in colonization by meso- or macrofauna when compared to the 1 cm bags ($p=0.571$ and $p=0.880$).

The results of this preliminary study led to the choice of the following mesh sizes to be used: 0.02, 0.25 and 10 mm. These sizes appeared to be the most suitable to distinguish between the contributions of the three size classes of the soil fauna. In fact, they are the same as those used for many years by the first author in pedobiological studies in temperate forests (Beck et al. 1988). The same mesh sizes were also proposed during a meeting of the German Working Group on Mesofauna (Ahrens et al. unpublished), based on the experiences of seven working groups, covering mainly forest and agricultural sites.

The experiment also allowed to determine the sample numbers necessary to evaluate decomposition effects by differentiation of the access of the target animals. The standard error values (in % of the mean) of the faunal abundances in ten bags of each mesh size were calculated as follows: 11.6 % for mesofauna in the 0.25 mm bags; 27 % for macro-, 10.5 % for mesofauna and 11.2 % for all animals in the 1 cm bags. Single macrofauna groups showed values of 19.6 % (Diplopoda), 21 % (Isopoda), 51 % (adult Coleoptera), 56 % (Araneae) and 90 % (Formicidae) in the large bags.

Ten bags of the intermediate mesh size, collected and evaluated at every retrieval date along the decomposition period under study seem to give reasonably accurate measures of colonization by mesofauna. However, the standard errors for macrofauna in ten bags of the large mesh size are still high, representing the extremely patchy distribution of soil animals in the tropics, and thus we decided to use 14 bags of each mesh size and retrieval date in the main study.

All established sampling methods together with work plans and detailed step-by-step instructions for many procedures are being compiled into a "handbook", accessible via internet from our homepage: http://www.cenargen.embrapa/~mgarcia/shift. Up to now, manuals for the study of soil biology are available only in German and for temperate regions (e.g. Dunger and Fiedler 1997; Schinner et al. 1996), or the methods described for the study of the soil fauna are, in our view, not sufficient (e.g. Anderson and Alexander 1993).

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Table 1: Number of animals per litter bag with different mesh sizes (only groups with more than 10 individuals summed over all bags are listed) exposed for 4 weeks and extracted with a Kempson apparatus; n = 10; all numbers are rounded to the next full individual.

mesh size [mm]	0.02	0.25	0.5	10	all bags
animal group	mean number of individuals per bag ± standard deviation				total number
Araneae	0 ± 0	0 ± 0	3 ± 3	4 ± 6	67
Pseudoscorpiones	0 ± 0	1 ± 1	5 ± 2	8 ± 10	140
Isopoda	0 ± 1	0 ± 0	6 ± 5	7 ± 5	136
Diplopoda	0 ± 0	1 ± 2	4 ± 4	4 ± 2	81
Coleoptera adult	0 ± 0	1 ± 2	1 ± 1	0 ± 0	21
Macrofauna	0.7 ± 0.8	3.1 ± 3.5	18.1 ± 8.5	22.5 ± 19.3	445
Coleoptera immature	0 ± 0	1 ± 1	1 ± 1	1 ± 1	23
Acari	16 ± 10	160 ± 56	105 ± 42	181 ± 70	4617
Collembola	14 ± 14	139 ± 80	102 ± 62	69 ± 30	3233
Copepoda	0 ± 0	3 ± 3	4 ± 4	1 ± 2	85
Mesofauna	30.3 ± 17.3	302.1 ± 111.1	211.8 ± 89.3	252.8 ± 83.9	8346
Diptera immature	1 ± 1	1 ± 1	1 ± 1	2 ± 2	44
Diptera ad.	1 ± 1	1 ± 1	0 ± 1	0 ± 0	31
Homoptera	0 ± 0	0 ± 0	1 ± 1	1 ± 1	24
Formicidae	4 ± 10	6 ± 14	9 ± 19	7 ± 18	256
Protura	0 ± 0	1 ± 2	1 ± 2	1 ± 2	35
Total	36.6 ± 17.3	314.3 ± 115.9	242.6 ± 95.8	285.7 ± 100.9	8791