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Fungi flora inventory in soils under different agrosystems along a gradient of cultivated fields in Oumé region, West Central Côte d'Ivoire

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1 SUMMARY

Soil samples were collected using a grid system spanning from natural forest areas to more degraded cultivated zones in order to monitor biodiversity changes along a gradient of land-use intensification. Organisms concerned were the microscopic fungi. Soil samples were collected at 3 and 6 m radius circles around each point. Overall, 107 points were sampled of which 40, from the 8 land use types were analysed. Fungi were identified based on both macroscopic and microscopic morphological characteristics. Total microflora identified from the points showed that 4-year-old teak plantations were less rich in fungi, whereas 10-year-old teak plantations were richer in fungal flora. When grouping the communities into morphological units or genera, it appears that genera such as *Aspergillus*, *Penicillium*, *Trichoderma* and *Fusarium* were the most frequently encountered and were present across the landscape. However, other fungi genera, such as *Sclerotium*, *Acremonium*, *Geotrichum*, *Pythiopsis*, *Phytophthora*, were rarely found in this environment.

2 INTRODUCTION

Soils are highly complex systems, with many components playing diverse functions mainly due to the activity of soil organisms (Chiang and Soudi, 1994). The role of the diverse soil organisms can be beneficial or not beneficial and soil fungi fulfil both roles and are ubiquitous. Fungi belong to the heterotrophic and eukaryotic thallophyte group; that is, fungi having a nucleus similar to higher plant species. This formally distinguishes them from bacteria and actinomycetes that are prokaryotes (Dommergues and Mangenot, 1970; Davet, 1996).

In the beginning of the 20th century, some authors doubted the existence of fungi in soils. According to them, the development of the mycelium observed on media containing soils simply proved the presence of dormant spore species contained in organic debris of the soil suspension, or originating from atmospheric contaminants. Today, the existence of soil fungi, which varies according to soil type, vegetation structure, climate, is well-agreed upon, in spite of the fact that its activity, biomass and composition are in some cases difficult to understand (Dommergues and Mangenot, 1970). A study of soil microbial characteristics can be based on: (1) the interactions between soil and microbial communities, (2) the interactions between the

microbial communities, (3) the interactions between microbial communities and the vegetation structure. A number of fungi species are known for their role in the agro-ecosystem either because they are associated with crop diseases or because they are used in the biological control of pest or involved in organic matter decomposition. In Côte d'Ivoire, very few studies were devoted to the soil fungi in an ecological and sustainable agriculture approach. This situation justifies, in part, the interest of this study. Specifically, the study aims at making inventory of soil fungi, especially the plant pathogenic and the saprophytic-decomposer groups in agro-ecosystems spanning from forest areas to more degraded mixed-crop fields in the Oumé area, west-central Côte d'Ivoire.

3 MATERIALS AND METHODS

3.1 Study site and sampling: The study area is located in the Department of Oumé (West central Côte d'Ivoire) and is defined by the following coordinates: 6°31'14,47 and 6°30'09,88' N; 5°30'05,20 and 5°28'59,98' W. The study site covers 4 km^2 and spans from Téné state forest to the rural domain. The specific location was selected such that a variety of land uses exists within the window (or sampling frame) (figure 1). The land-use systems present are natural forest, secondary forest, cocoa plantation, mixed-food cropping, recurrent fallows and multi-species plantations. The standardized sampling method agreed upon by the Conservation and Sustainable Management of Below-Ground Biodiversity Project was used to sample .soil. At each sampling point, six sub-samples were taken at 3 and 6 m radius circles. In total, 107 points were sampled of which 40 (five points per land-use type) were analysed in the laboratory. Upon collection, soil samples were freed of plant debris and coarse elements were removed by sieving. Then in the laboratory, samples were homogenised and stored in transparent 2-liter plastic bags in a well ventilated at room, at temperature between 25 to 35 °C or at 14 °C for later analyses.

3.2 Fungi isolation using suspension-dilution technique: The objective was to isolate, not only fungi mycelia from soil, but also to quantify them. Soil sample (10 g) previously passed through a 1.5 mm mesh screen was transferred into an Erlenmeyer flask to which 90 mL of distilled water was added. This made up the undiluted primary suspension (D0). The suspension was then homogenized using a magnetic stirrer for 30 min at 100 rpm. Then, 1mL aliquot was drawn and put into a culture tube containing 9 mL of sterile distilled water: This was the 10^{-1}dilution suspension. This process was repeated to obtain the 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions.

For all samples isolated, using this technique, the 10^{-3} dilution was used after preliminary trials, revealing that at this dilution, the bulk of the colonies can be clearly identified. Furthermore, a 1 mL soil suspension was drawn using dilution technique and put into a 9-cm-diameter Petri dish containing a medium maintained under suffusion at about 45 °C. Acidified malt medium (20 g of malt extract, 20 g of agar and 250 mg citric acid added after sterilization in an autoclave) was first selected because it has the advantage of extracting maximum fungi. Petri dishes were incubated in the oven at 25 °C in the dark (5 Petri dishes per dilution). After three (3) days, colonies that appeared were counted, described and observed under a microscope. They were then transferred individually into a new medium and purified for identification. The culture media used were mainly PDA (dehydrated potato flakes: 20 g, glucose: 20 g, agar: 20 g in 1000 mL of distilled water) and Sabouraud (peptone: 10 g, glucose: 20 g, agar: 15 g, chloramphenical: 250 mg in 1000 mL of distilled water).

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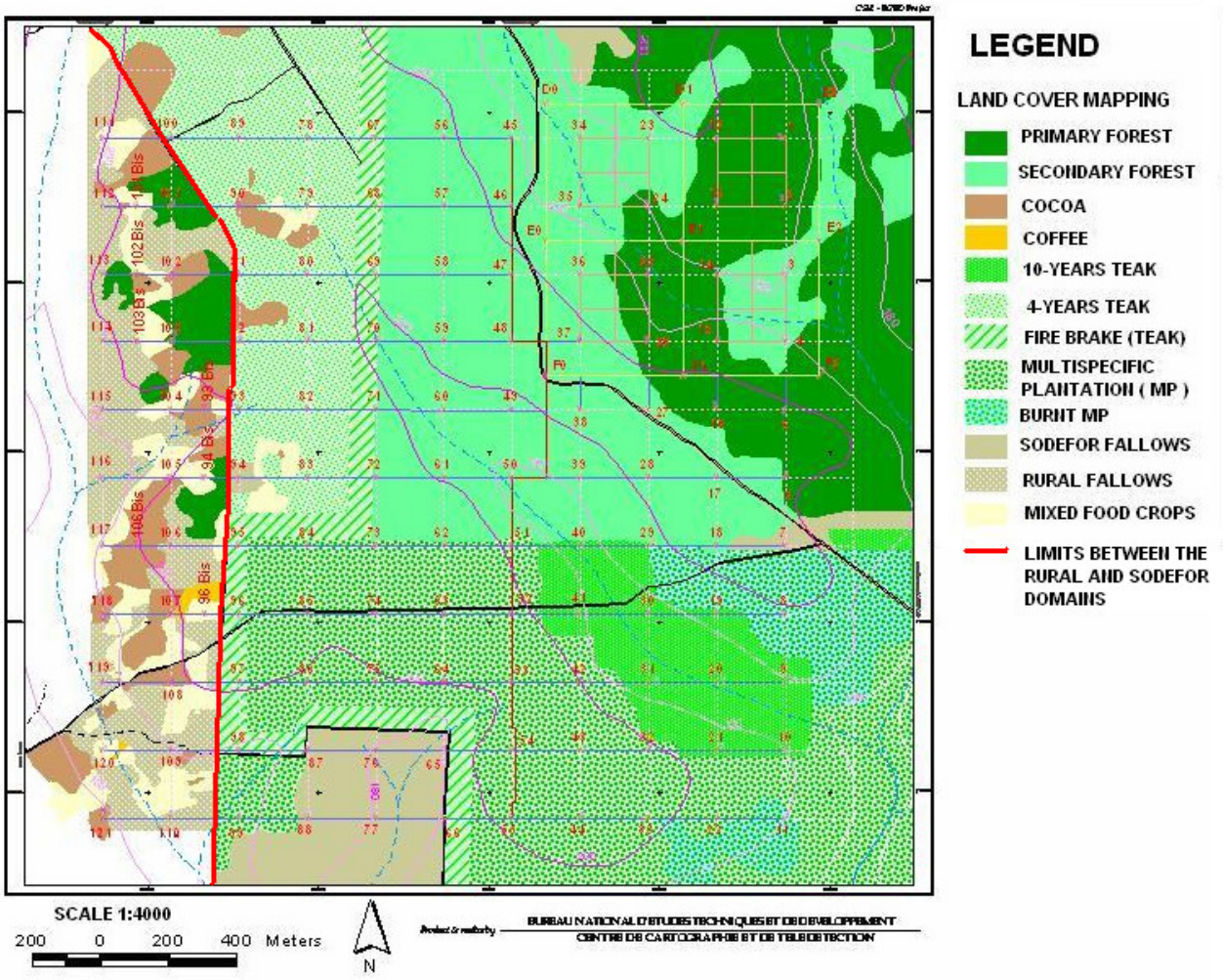

Figure 1: Map of sampling area showing the land use types (from CSM-BGBD project maps)

3.3 Isolation using soil particles or soil washing method: Soil sample (10 g) was suspended in water, agitated and passed through a 0.5 mm mesh screen. This process was repeated several times. Soil particles were dried on a sterile tissue paper and later transferred into Petri dishes containing 2% water-agar amended with 250 mg L^{-1} of chloramphenical. The Petri dishes were incubated for 24 to 48 hours at 25 °C. The frequency of particle colonisation was determined; the colonies were then purified.

The suspension-dilution and the washing techniques were used on the basis of the quantitative and qualitative approaches. The quantitative approach consisted of to determine the relative quantity of fungi per gram of soil (Tello Marquina *et al*., 1980). The parameter allowed the quantification and comparison of population density from a given ecological zone to the other. It is based on the principle of CFU (colony forming unit). The use of the qualitative approach was recommended in this study. It consisted noting to report the type of isolated fungi. Hence, it considers the diversity within the observed population. Identification of species or groups, in this study, was possible with several keys (Barnett, 1969; Barnett and Hunter, 1972; Booth, 1971; Burgess *et al*., 1994; Gilman, 1971).

3.4 Statistical analysis: Arithmetic means have been obtained. The significance of difference was tested by one-way analysis of variance. Comparison of means was made by the Student Newman-Keuls test at 5 % level, using Statistica 7.1.

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4 RESULTS

For all the 8 land-use types, the flora counts were about 10^4 CFU g^{-1} of dry soil (figure 2).

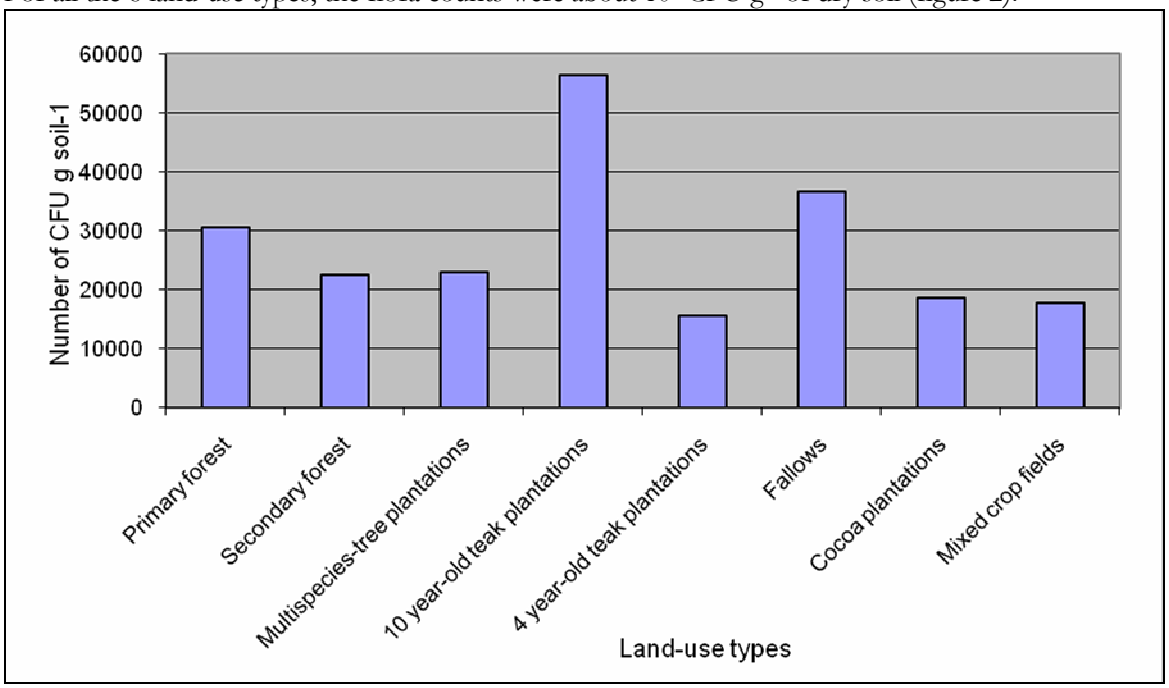

Figure 2: Overall distribution of total fungi flora across various land-uses

The comparison of means, with the Student Newman-Keuls test, discriminated 4 statistical homogeneous groups of soils (land-uses). The first consists of 10-year-old teak plantations that contained higher fungi colonies; the second includes fallows and primary forest; the third are formed by primary forest, multi-species-tree plantations and secondary forest. Multi-species-tree plantations, secondary forest, cocoa plantations and 4 year-old teak constitute the fourth group. In this inventory, some groups/genera with important frequencies were identified (Table 1). These included *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp. and *Fusarium* sp. These groups of fungi were ubiquitous in most of the areas studied. However, *Fusarium* sp. was not isolated from the 10-year-old teak plantations and neither was *Trichoderma* in soils under fallow. Some fungal groups were rarely observed across the landscape. This was the case for *Spicaria* which was localised in the 10-year-old teak plantations, the sphaeropsidales group in cocoa plantation soils as well as *Pythium* and *Geomyces* in the primary forests. *Gliocladium* was isolated only from the multi-species plantations and 10-year-old teak plantations.

Table 1: Frequency and diversity of some fungal genus isolated across various land-use types in Oumé region, Côte d'Ivoire.

Morphological group/genera	PF	SF	MP	TK10	TK4	CC	F	MC
Fusarium	10	4	8	0	2	6	1	4
Trichoderma	11	7	3	6	1	8	0	1
Penicillium	10	4	6	4	2	26	7	10
Aspergillus	9	9	8	10	5	8	12	9
Gliocladium	0	0	1	2	0	0	0	0
Pythium	1	0	0	0	0	0	0	0
Other Phycomycetes	18	0	2	4	0	3	4	4
Other fungi	10	5	5	5	6	8	13	10

PF: primary forest, SF: secondary forest, MP: multispecies plantations, TK10: 10 year-old teak plantation, TK4: 4 year-old teak plantations, CC: cocoa plantations, F: fallows, MC: mixed crop fields.

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Focusing on some groups that have agronomic or ecological importance, we notice that the number of isolates of *Fusarium* sp., *Trichoderma* sp. and the phycomycetes (including *Pythium* genus) don't have the same dynamic in the different land-use types (figure 3).

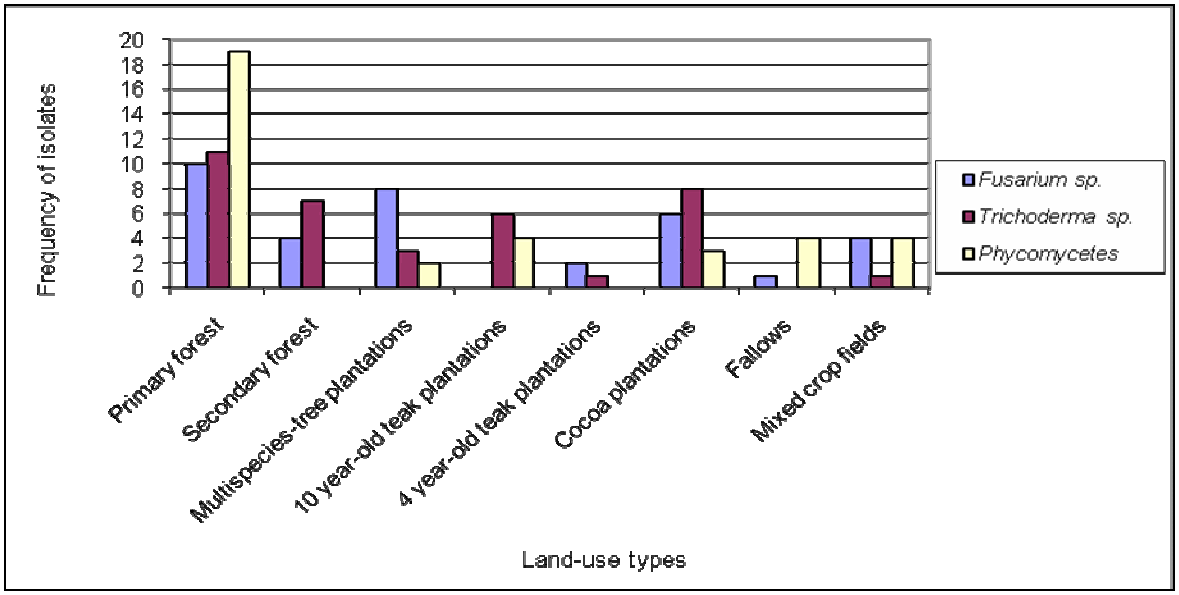

Figure 3: Frequency of isolates of Fusarium sp., Trichoderma sp., and Phycomycetes across the land-use types

Fusarium sp was more abundant under primary forest and the areas that were the least endowed with this taxon were the 10-year-old teak plantations. The primary forest was also the richest land use in members of the phycomycetes group. The 4-year-old teak plantations were found to be the poorest in terms of the presence of this fungal group. For Trichoderma sp., a taxon presenting a good potential for biological control agents, the primary forest once again remained the richest in the population of this fungus. However, no Trichoderma sp. was found in the fallow fields

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5 DISCUSSION

Fungi population levels obtained by the method of suspensions and dilutions, about 10^4 CFU g^{-1} of dry soil, are similar to those obtained by Abo (2006) in other soils of Côte d'Ivoire. Total fungal flora, determined in his study, in soils under cotton cultivation, varied from 4.6. 10^4 to 7.4 10^4 CFU g^{-1} of dry soil. Results from this study showed that the different land-uses were, quantitatively and qualitatively, variable in soil fungi. The ten-year-old teak plantations, which represent an intermediate level of intensification, had the largest number of fungal cells, followed by fallow and primary forest. However, areas that were the richest in terms of diversity were primary forest and multi-species plantations. These systems are characterised by a high floristic diversity, which could have influenced the greater fungal diversity. Given the relatively rich floral composition of fallows, it unexpectedly had the least number of fungal types isolated. Secondary forest, teak and cocoa plantations, as well as mixed crop fields, had all the same number of fungal types isolated. Although a relationship can be established between abundance and diversity for the other land-use types, this was not the case for the mixed crop fields. The latter land-use, which was quite depleted in total flora, appeared to be rich in terms of fungal genera.

It is known that the rhizosphere greatly influences the microbial population type associated with it. The relationships between microbial populations and their biotope or, indirectly, between themselves depend on the type of carbon substrate (Youssef and Heitefuss, 1983; Garland and Mills, 1991; Roger and Garcia, 2001). Therefore, it is understandable why mixed crop systems, or even fallow fields, housed highly diversified fungal groups. However, it appears clearly that this relationship was not true for the 4-year-old teak plantations which were

devoid of Phycomycetes fungi. Additionally, there was the localisation of some fungal genera, such as *Pythium* in Primary forest. Thus, it is possible that additional ecological elements are probably responsible for this multiform expression of the fungi flora. The functional groups, within the fungi population described, will only be definitely identified by using other protocols. For now, it could be said that genera containing known plant pathogens were isolated. Further characterization is needed to go up to species level. Fungi, such as *Trichoderma* which species are involved in biological control of plant pathogens (Camporota, 1985 ; Ubalua and Oti, 2007), were also isolated. But the fungus was not found in fallow field due to the probable absence of pathogenic fungi that are attacked by *Trichoderma* species. Of course, for the use of *Trichoderma* species in a context of sustainable agriculture, their study in the local environment must be continued and deepened.

6 CONCLUSION

The results of this work show that important fungi richness was identified within the different ecological units investigated. This richness was found to change from one area to the other across the land use types. The 10-year-old teak plantations, which represent medium level of intensification, had the largest number of fungal propagules, followed by fallow and primary forest. The frequency of isolates of *Fusarium* sp., *Trichoderma* sp. and the phycomycetes that have agronomic or ecological importance varied, but doesn't follow automatically the evolution of stability or degradation of the ecological profile displayed by the different land-uses.

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