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INFLUENCE OF LAND USE ON THE DISTRIBUTION AND DIVERSITY OF *PYTHIUM* SPP.

[INFLUENCIA DEL USO DEL SUELO EN LA DISTRIBUCION Y DIVERSIDAD DE PYTHIUM SPP.]

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SUMMARY

The distribution of *Pythium* species across land use gradient was examined in two agroecological zones in Kenya. The two zones were selected owing to their unique characteristics and locations. Soil samples were collected along a transect from the forest through to the cultivated land. The sample plots were established at fixed intervals of a distance of 200m apart to avoid auto-correlation *Pythium* propagules were activated to germination using baiting techniques, and the mycelium baited was cultured to obtain axenic culture for identification. Based on morphological characteristics, a total of 32 species of *Pythium* were found at the Embu study site, with soil under Napier having the most diverse population and that under maize the least. The Taita site yielded 12 *Pythium* species, with the highest diversity in cropped plots and the lowest in Natural forest. Species abundance increased with increasing ecosystem disturbance while diversity increased with decrease in disturbance. All the species isolated from the Taita site were also sampled from the Embu site except *P. helicoids*, *P. periplocum* and *P. grandisporangium*. The common pathogenic species *P periplocum, P. hypogynum and P. spinosum* were isolated mostly from croplands confirming the effect of host occurrence on the distribution of *Pythium* species.

Key words: Land use types; *Pythium;* distribution.

INTRODUCTION

The genus *Pythium* comprises of 120 described species which vary in their pathogenicity and crop hosts. The species that infect plants are ubiquitous in soil. The wide distribution and host range of this genus demonstrate its ecological importance and its impact on many human activities. The fungus is known to cause a group of diseases such as 'damping-off' of seedlings, root and stem rots, and blights in many plants which vary in their pathogenicity and crop hosts. Significant loss of soil diversity or species richness has been found to be influenced by agricultural intensification and use of inputs such as fertilisers and cropping systems. For example intensive mechanical tillage shows a significant negative effect on soil biota. On the other hand removal of this disturbance is associated with increased weed development and retention of residues which stimulate diseases caused by *Pythium* that are retained in them leading to increased use of herbicides and pesticides (Warlde's, 1995). This study was conducted to make inventory of the diversity and distribution of *Pythium* spp. in various land use systems in Irangi and Ngangao Forests in Embu and Taita respectively.

MATERIAL AND METHODS

Description of the study site

The study areas are located in Embu and Taita Districts. The Embu study site is along the lower slopes of Mt. Kenya where Irangi Forest is located. The area below the forest belt is utilized for agriculture with tea on the upper slopes and coffee on the lower slopes. The rest of the area is under subsistence agriculture with agro forestry being widely practiced in many parts as a means of soil and water conservation.

The rainfall pattern is bimodal with two rainy seasons - long rains in April to June and short rains in November and December. The average annual rainfall is about 1495 mm. Agricultural management practices are characterized by the use of relatively high levels of inputs, such as manure, fertilizers and pesticides. These inputs are particularly used for maize, tea, coffee and irrigated horticultural crops.

The second benchmark site are the Taita Hills which cover an area of 1000 km^2 and form the northernmost part of the Eastern Arc Mountains. The mean annual rainfall ranges from 500 mm in the lowlands to over 1500 mm in the upper mountain zone. There are two rainy seasons in the area, March-May/June and October-December. The land use in this region is dominated by intensive agriculture with extensive agriculture and grazing dominant on the foothills and plains surrounding the hills.

Soil sampling

Soil samples were collected using a systematic grid which were 200m apart to avoid auto-correlation. Three windows were selected in two locations within Embu District. This gave a three-stage sampling design (multi-stage) with systematic random samples taken at Window level as first stage, Land-use level as second stage and sampling points as third stage based on Chatfied (1988). At each sampling point, three samples were collected in manilla bags at various depths between 0-10 cm, 10-20 cm and 20-30 cm. The soil samples were stored at room temperature before isolation of fungus.

Isolation of *Pythium* spp.

Soil samples from depths 0-10 cm, 10-20 cm and 20-30 cm were screened separately. Moist soil incubated at room temperature for three days. Sub-samples of 0.5 g of pre-moistened soil were then placed in sterilized glass test tubes and mixed with 20 ml of sterilized water. *Pennisetum clandestinum* (Kikuyu grass) leaf blades were sterilized with sodium hypochlorite (1% for 30s) and placed in the test tubes for incubation. The samples were incubated for three to five days at 25°C in the dark. Infected tissues were transferred into sterile water containing antibiotics (chloramphenicol) for a few hours. The growing mycelium was verified directly by water mounts under a microscope or transferred from the bait to the isolation media CMA (Corn meal agar) amended with chloramphenicol (20mg L-1) and Benomyl (10 mg L-1) (Tsao *et al*. 1983; Gams *et al*. 1998; Edena *et al*. 2000). Those species that produced submerged cultures on CMA were cultured on Principal Component Analysis for further screening. Quantitative assays on distribution were done using bait tissues among replicates in each soil sample. There were three replicates for each sample. Morphological characteristics were used to categorise the isolates into groups, which were then screened to species level using Van Der Plaats Monograph (1983).

RESULTS

Thirty two species of *Pythium* were recovered from Embu soils. The diversity and abundance varied with land use as shown in Table 1.The most diverse land use type was napier grass with nine species and maize had the least. Forest soils recorded six species while fallow and coffee LUT recorded five species. The most common species were *P sylvaticum*, *P. flovoense*, *P. astrocodes* and *P. macrosporum.* These species were recovered mainly from soils collected from the forest and fallow fields. *P. flovoense* was isolated from natural forest, coffee and fallow plots while *P. astrocodes* was recovered from natural forest, fallow and maize fields. *P. sylvaticum* occurred in planted forest and tea plots, while *P. macrosporum* was sampled from soils under planted forest, napier and maize. *P. spinosum* occurred in coffee and napier grass.

Table 1. Abundance and diversity of *Pythium* species in Embu.

Land Use Type	Diversity (%)	No. of species
Natural forest	44	*P. grandisporangium* *P. flovoense* *P. ostrocodes* *P. aphanidermatum* *Pythium* sub 4 *Pythium* sub *42*
Planted forest *(Vitex kenienis)*	44	*P. sylvaticum* *P macrosporum* *P heterothallicum* *Pythium* sub 46 *Phythium* sub 48 *Phythium* sub 50
Camellia sinesis (Tea)	51	*P. sylvaticum* *Pythium* sub 9 *Pythium* sub 12 *Pythium* sub 59 *Pythium* 3A/3C
Coffea arabica (coffee)	32	*P. spinosum* *P. flovoense* *P. buismaniae* *P. papillatum* *Pythium* sub 32
Pennisetum purpureum (Napier)	32	*P. pulchrum* *P. graminicola* *P. splendens* *P. macrosporum* *P. spinosum* *P. ultimum* *Pythium group G* *Pythium group C* *Pythium* sub aerial 36
Fallow	48	*P. anadrum* *P. orthogonon* *P. flovoense* *P. ostrocodes* *P. hypogynum*
Zea mays (Maize)	31	*P. ostracodes* *P. coloratum* *P. macrosporum* *Pythium* sub 20

Diversity (p= 0.810) and richness (p=297) of *Pythium*,

Table 2. Relationship between land use and distribution of *Pythium* in Embu.

Land use	Mean diversity	Mean richness	Mean evenness
Coffee	0.026	0.444	0.025
Fallow	0.000	0.833	0.018
Maize	0.046	0.500	0.025
Napier grass	0.085	0.619	0.039
Natural Forest	0.032	0.750	0.018
Planted Forest	0.066	0.778	0.019
Tea	0.060	0.727	0.020
P value	0.810	0.297	

Principle component analysis showed that species were attracted to different land use types. Factor 1 which accounted for 33.94 % of the total variation could therefore be host factor. Factor 2 separate LUTs with low organic matter with those LUTs with high organic matter. As intensification reduce from napier towards fallow, coffee, tea, plantation and natural forest, *P. graminicola* and *P.spinosum* species were lost which translated to reduction in pathogenecity. Absence of disturbance lead to increased counts of *P. graminicola* and *P. spinosum. P.macrosporum* soils under natural forest and can therefore be used as a bio-indicator of land degradation. It is clear that forest disturbance affects mostly *P .graminicola*, *P.spinosum* and *P. hypogynum* increasing their abundance and diversity, Fig 1. These are pathogenic species.

In Taita 12 *Pythium* species were isolated and the most common species were *P periplocum and P. spinosum* which were recovered from planted forest, shrubland and cropland, Table 3.

All the species isolated from Taita site were also sampled from the Embu site except *P. helicoids*, *P. periplocum* and *P. grandisporangium*.

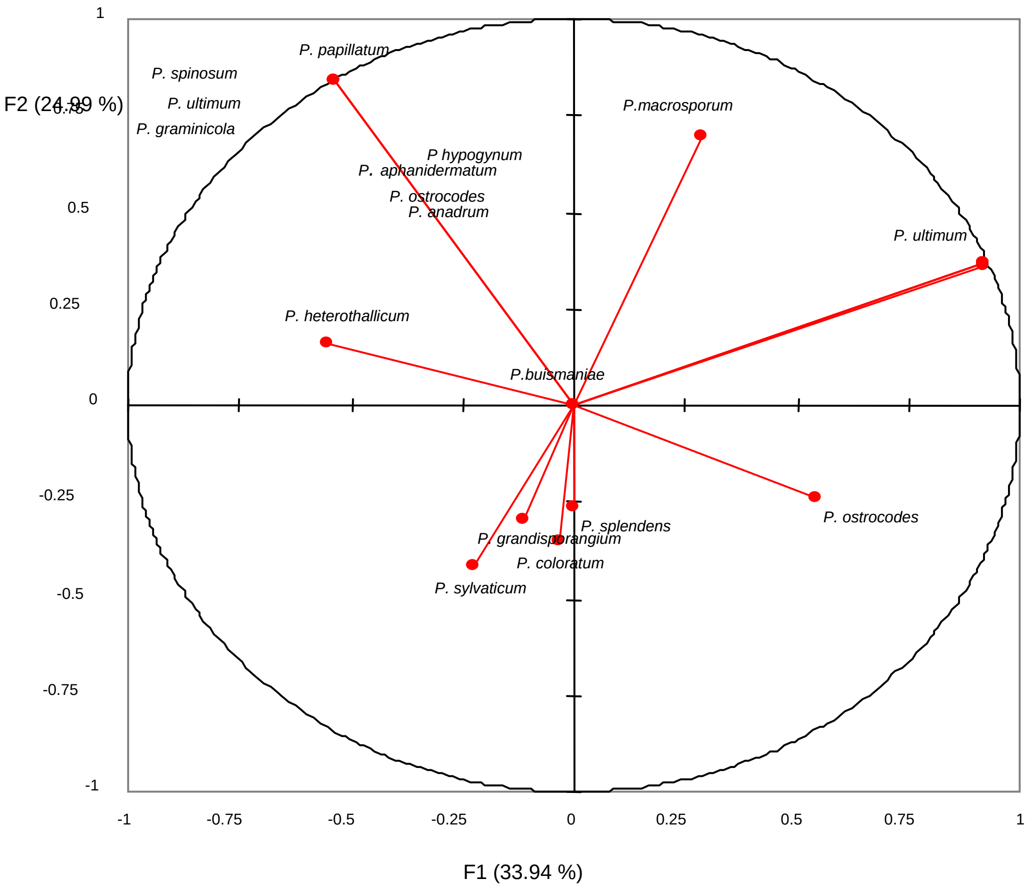
Species diversity ($p \leq 0.030$). and richness ($p \leq 0.015$) was significantly influenced by land use type. Fallow was the most diverse and the most rich land use type (0.256) followed by natural forest (0.087), horticulture (0.069), while maize, nappier and coffee have mean diversity of 0.000. Indigenous forest and coffee recorded the least abundance, Table 4.

According to the PCA results crop lands and indigenous forests were separated from fallow since they supported low host diversity. The second axis separated LUTs with low organic matter with those LUTs with high organic matter. As intensification decreased from horticulture to maize, fallow, indigeneous forest *Pythium flovoescence*,and *P. helicoides* are lost while *P. ultimum* and *P. sylvaticum* species increased.

Three major groups emerged as LUTs were separated into three distinctive clusters. The first two factors accounted for 77.4 % of the total variation. The first group consists of P.flovoecense and P.helicoides with high negative correlation and the second group is for the P. periplocum, P. sylvaticum and P. hypogynum with low positive correlation, and the while the third group comprises of P.ultimum and P. sylvaticum with moderate negative correlation for both factor 1 and 2.

Table 3: Abundance and diversity of *Pythium* species at Taita.

Land use type	Abundance (%)	Species
Natural forest	53	*P. helicoids* *P. ultimum*
Planted forest	50	*P. periplocum* *P. spinosum* *P. flovoense* *P. grandisporangium* *Group 3C*
Shrub land	67	*P. periplocum* *P. spinosum* *P. hypogynum* *P. aphanidermatum* *P group G* *Group 3C*
Crop land	28	*P. periplocum* *P. spinosum* *P. flovoense* *P. ultimum* *P. sylvaticum* *Group 3B* *Group 3C*



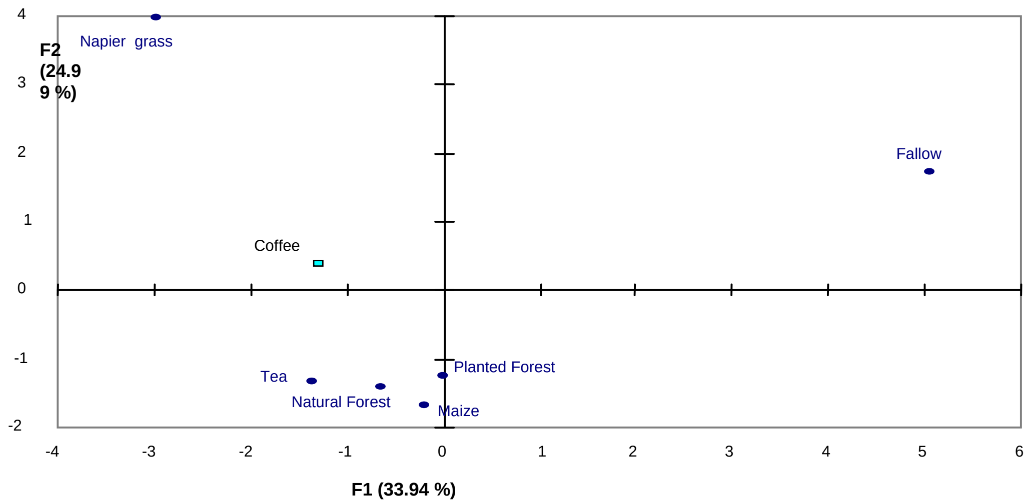
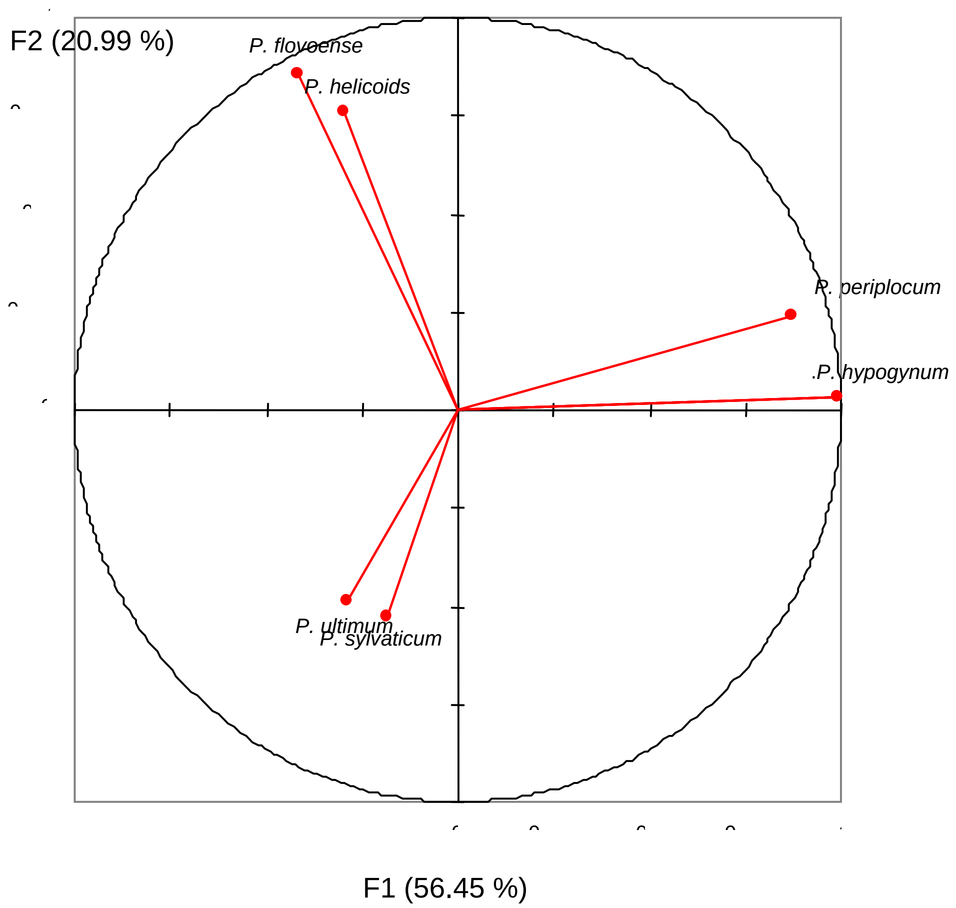


Figure 1: Principal Component Analysis separating effects of land-use types on the distribution of *Pythium* spp in Embu. Variable and observation axes F1 and F2 = 58.93%



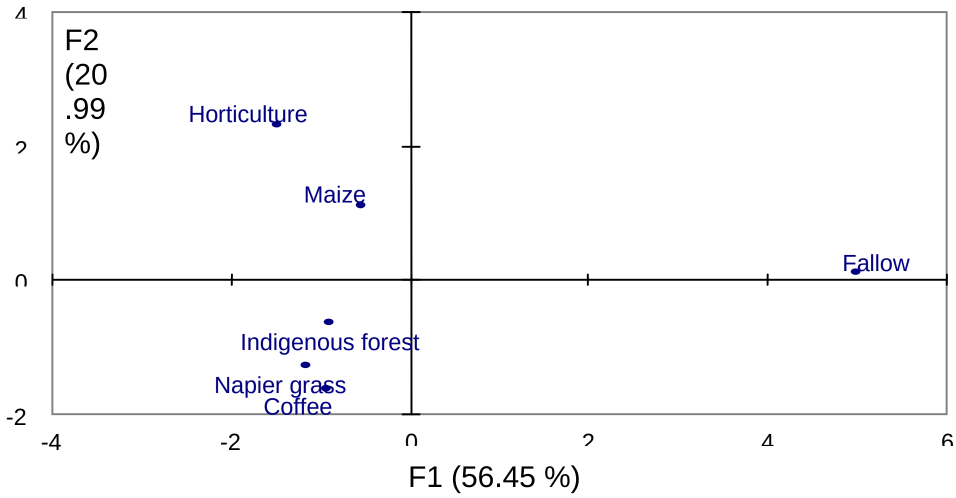


Figure 2: Principal Component Analysis separating effects of land- use types on the distribution of *Pythium* spp in Taita. Variable and observation axes F1 and F2 = 77.44%

Table 4. Relationship between land use and distribution of *Pythium* in Taita.

Land use type	Mean diversity	Mean richness	Mean evenness
Coffee	0.000	0.700	0.0667
Fallow	0.256	1.143	0.0254
Horticulture	0.069	0.700	0.1000
Natural forest	0.087	0.250	0.1250
Maize	0.000	0.375	0.0612
Napier grass	0.000	0.625	0.0983
P value	0.030		0.015

DISCUSSION

Pythium species was widely distributed in all land use types but the diversity in unperturbed systems was high while the number of individuals (abundance) in each species was relatively low. This agrees with the findings of Poole (1974). This can be explained in terms of pathogenicity. In cultivated or disturbed environments the pathogenic species thrive and out grow the saprophytic species as observed with *P periplocum, P. hypogynum and P. spinosum* in planted forest, shrubland and cropland presented by the ordinations.

Soil fungistasis may have played a key role in the absence of baited mycelium in some points especially from forest soil where high diversity and species population was expected. There are complex biological relations between different soil organisms and higher plants on which they may be parasites (Hawksworth, 1983). Hendrix et al. (1986) showed that soil conservation management practices (minimum tillage, crop rotations, stubble retention on the soil surface) improved soil structure organic matter, microbial population, especially in the surface soil layers tended to be dominated by fungal species. In this study presence of plant influenced distribution and abundance of *Pythium* species.

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