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Impact of Land Use Types on Spore Abundance of Arbuscular Mycorrhizal Fungi in the Humid Tropical Rainforest, Southeast Nigeria

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Abstract
The study assessed spore populations of the arbuscular mycorrhizal fungi (AMF) in selected land use types (LUT) in Obehie, Asa area of Abia State, Southeast Nigeria. Soil samples were collected at 0 – 15cm depth from four LUT (cassava, vegetable, yam and fallow fields) using a soil auger. Soil properties and mycorrhizal spore populations were determined in the laboratory and the data analyzed. The result shows that, the soil pH of the fallow LUT was strongly acidic (pH = 5.4), while those of the cassava (pH = 5.7), vegetable (pH = 5.5) and yam (pH = 5.9) LUT were moderately acidic. Organic C was highest in the fallow field (15.25 g kg^{-1}), whereas the highest contents of total N (1.19gkg^{-1}) and available P (69.91mgkg^{-1}) were recorded under the vegetable LUT. Concentrations of the exchangeable base cations occurred in the decreasing order thus: Ca > Mg > Na > K and generally highest in the vegetable farm compared to other LUTs. Spores of the AMF varied significantly ($P < 0.05$) across the LUT, being more abundant in the cassava LUT (237 spores 100g^{-1} soil) followed by the fallow (160 spores 100g^{-1} soil), yam (126 spores 100g^{-1} soil) and vegetable (112 spore 100g^{-1} soil). Spore abundance of AMF showed a significant ($P < 0.05$) positive correlation with organic carbon (r = 0.640*). Soils of cassava fields should therefore be considered as viable sources of soil-borne AMF inoculum in the studied area.

Keywords: Abundance, arbuscular mycorrhizal fungi, humid tropics, land use types, spore

Introduction

Arbuscular mycorrhizal fungi (AMF) are distinct group of soil microorganisms belonging to the genus Glomeromycota, which invaginate plant cells, forming a direct physical link between soil and plant roots (Barea and Jeffries, 1995). Symbiosis of the AMF and roots of plants is one of the oldest and most widespread beneficial soil microorganisms-plant interactions of agricultural significance. The AMF associate with a vast range of plant species and are more common than other types of mycorrhizal associations (Allen *et al*., 2003). At least 80 - 90% of all terrestrial plants and 90% of agricultural plants are known to exhibit arbuscular mycorrhizal dependency (Smith and Read, 2010). Plants capable of establishing mycorrhizal associations enjoy numerous benefits from mycorrhizal colonization compared to non-mycorrhizal plant species. The AMF essentially enhance plant's uptake of nutrients, especially phosphorus, through the fungal hyphae which helps to extend the root absorbing area of colonized plants. In addition, they contribute to increased plant uptake of water (Choi *et al.,* 2018; Smith *et al*., 2010) and improved tolerance to biotic and abiotic stress (Camenzind *et al*., 2018) as well as offer protection against plant diseases (Linderman, 2000). Mycorrhizal spores are common in agricultural soils (Smitha and Read, 2010) and their abundance is often approximated to the fungal populations since spores account for a key reproductive structure of the AMF (Egboka *et al.,* 2022). The sporulation and abundance of the AMF in soil may be generally affected by biotic factors including dominant vegetation types and abiotic factors such as climatic and soil properties.

Land use refers to the arrangements, activities and inputs people undertake in a given piece of land to protect, change or maintain it (Ufot *et al*., 2016). The land can be subjected to agricultural, recreational, engineering or aesthetic uses. Agricultural land use types typically involve lands devoted to agricultural practices and may include cultivation of open field with crops such as fruits, vegetables, grains, fibers, ornamentals and nursery plant materials or even fallow lands. Different plants and plant communities in natural and agricultural ecosystems can greatly affect the abundance of AMF in the soil (Dalpe *et al*., 2000; Jefwa *et al*., 2006). Adding a non-mycorrhizal host plant to cropping systems can pose strong negative effect on

AMF abundance and root colonization, thereby affecting nutrient uptake and the consequent crop yield (Douds and Millner, 1999). Oehl *et al.* (2003) noted significant effect of land use intensity on spore abundance and diversity of the AMF in Central Europe. Gonzalez-Cortes *et al.* (2012) found that, conversion of forests to maize fields reduced spore abundance and species diversity of the AMF. According to Cardozo-Junior *et al*. (2012) the presence of legumes and grasses increased spore population and species composition of the AMF over extended growing season. While species-rich communities of AMFs were observed in different natural and perennial ecosystems such as tropical forests, fewer communities were recorded in arable lands (Snoeck *et al*., 2010). To assess AMF abundance, Cuenca and Lovera (2010) carried out an intensive collection of spores from soils of a shrub land ecosystem in Venezuela. Among other findings, they observed a variation in spore density of the AMF with different land use types.

Tropical soils of humid and sub-humid African countries are vulnerable to soil degradation (Agboola, 1987) due to high rainfall intensity and warm temperatures which encourages rapid decomposition of soil organic matter, leading to low soil fertility. To boost the fertility of the poor tropical soils, there is renewed interest in harnessing the potentials of soil-borne beneficial microorganisms which serve as natural bio-fertilizers. Among the promising soil microbiota that promotes soil nutrients availability and increases agricultural productivity in a sustainable manner is the AMF. However, being obligate biotrophs, the AMF survives only on their mycotrophic host plants (Birhane *et al*., 2012), which can pose variable effects on the sporulation ability of the organism. Investigation of the influence of vegetation types on abundance of the AMF in soils, therefore, becomes pertinent especially in the tropical rainforest regions, where plant species are most diverse than in any other ecosystem. Hence, the present study was undertaken in the humid tropical rainforest area of southeast Nigeria to assess the spore abundance of arbuscular mycorrhizal fungi as influenced by land use types.

Materials and Methods

Study area

The research was conducted on soils of Obehie, Asa in Ukwa West Local Government Area (LGA) of Abia State, Southeast Nigeria. The area is located within latitudes $4^{o}94'$ N and longitudes $7^{o}02'$ E of the equator with elevation of 73m above the sea level. The climate of Obehie, Asa area is humid tropical, having both wet and dry seasons. The wet (rainy) season starts in April and ends in November, while the dry season commences in December and ends in March, resulting in a typical bimodal rain distribution pattern. The average annual rainfall and temperature is about 2500mm and 29˚C, respectively. During the rainy season, humidity is high, but decreases in dry season. The vegetation of the area is typical of rainforest, characterized by multiple plant species arranged in layers. Plant species commonly found in the area include tree species such as *Elaeis guineensis* (Oil palm), *Cocos nucifera* (Coconut), *Musa sapientum* and arable crops such as *Manihot esculentus* (cassava), *Zea mays* (Maize), *Colocasia spp.* (Cocoyam), *Dioscorea spp.* (yam) and vegetables. The hydrology of the area is controlled by "Imo River" and agriculture is the major socio-economic base of the people of the study area.

Site selection and soil sampling

Following a reconnaissance survey of the area, cassava, yam and vegetables were recognized as prominent agricultural crops widely cultivated in Obehie, Asa community, hence the choice of the LUT. Using the soil auger, three (3) replicate soil samples were collected randomly at 0 - 15cm depth of soil from each of a cassava, yam, vegetable and fallow LUT within the study area. The soil samples (12 replicate samples in total) were carefully carried to the laboratory where they were processed for determination of both soil properties and AMF spore density.

Determination of soil properties and quantification of AMF spores

The physical and chemical properties of the soils were determined in line with standard laboratory methods: Particle size distribution (Gee and Or, 2002), Soil pH in a 1:2.5 soil-water suspension using the glass electrode pH meter (Thomas, 1996), Exchangeable acidity (H + Al ions) determined in 1N KCl extracting solution with 0.5N Na0H using phenolphthalein indicator by the titration method of Mclean (1982), Organic carbon estimated titrimetrically by Walkley and Black wet oxidation method (Mclean, 1982), Total nitrogen by micro-kjeldahl digestion method (Bremner, 1996), Available phosphorus extracted and determined calorimetrically by Bray and Kurtz No. 2 solution (Olsen and Sommers, 1982), Exchangeable base cations (Ca, Mg, K and Na) determined by extracting with 1N NH_4OA_C solution buffered at pH of 7, then exchangeable K and Na were estimated by flame photometry method while exchangeable Ca and Mg were determined by atomic absorption spectrophotometry (AAS) (Spark, 1996) and Effective cation exchange capacity was obtained by summation of the exchangeable acidity and exchangeable bases.

The wet sieving and decanting technique of Gerdemann and Nilcosin (1963) was adopted for estimation of the AMF spores in the soils. 100grams of each of the replicate soil samples was added to a large beaker containing 1000ml of distilled water. After one hour of settling, the mixture was separated by decanting the filtrate through four serial sieves ranging from 63µm to 500µm in diameter, arranged in the decreasing order. The sieving and decanting process was repeated thrice or more until a clear supernatant was obtained, and to enable the trapping of as many spores as possible. Materials retained in the two smallest sieves were then washed into centrifuge bottles with streams of water. Particles in the centrifuge bottles were placed in the machine and centrifuged for 2 minutes at 2000rpm.

After settling, the filtrate was decanted and sucrose solution (40%) added, then the mixture was again centrifuged for 1 minute at 2000rpm. The filtrate was then discharged into the two smallest sieves and materials retained in the sieves were washed into plastic bottles for examination of mycorrhizal spores. Spore counting was performed with the aid of a grid line Millipore and reported as number of spores per 100grams of soil.

Statistical analyses

Analysis of variance (ANOVA) was conducted on the generated data with the aid of GenStat computer package, edition 4.0. Differences among means were compared using the least significant differences at 0.05 level of probability while relationships between AMF spore abundance and soil properties were determined using the Pearson correlation analysis.

Results and Discussion

Soil properties of the land use types in the study area

The results of the soil properties are presented in Table 1. The soil pH of the studied land use types (LUT) indicates that soils of Obehie, Asa area are generally acidic. The mean pH values were not significantly ($P < 0.05$) different across the studied LUT and ranged from 5.4 in fallow land to 5.9 in the yam LUT, thus falling within the strongly acid to moderately acid soil pH class of Chude *et al.* (2012). Acidic nature of humid tropical soils has been attributed to high degree of leaching of base cations due to the characteristic high rainfall intensity of humid tropical environments (Ojanuga and Lekwa, 2005). Organic carbon content of the studied LUT varied significantly ($P < 0.05$) from low to moderate levels with the highest and lowest mean values recorded in the fallow (15.25gkg^{-1}) and cassava (6.23gkg^{-1}) LUT, respectively. The higher levels of organic carbon observed in the fallow field relative to other LUT may be as a result of the accumulation of organic materials in the form of litter falls in fallow systems (Egboka *et al*., 2021). Total nitrogen contents varied from 0.71gkg^{-1} (Fallow), 0.77gkg^{-1} (Cassava), 1.04gkg^{-1} (yam) to 1.19gkg^{-1} (vegetable) and were not significantly ($P < 0.05$) different across the LUT. These levels of total N were low to moderate compared to the ratings of Chude *et al*. (2012). According to Isirimah *et al*. (2003), soil degradation processes such as intense leaching, volatilization and erosion due to high rainfall, accounts for the key causes of nitrogen deficiency in tropical soils as nitrogen is very mobile in soil. Apart from the yam field, levels of available phosphorus were generally high in the studied LUT based on the critical limit of (> 20mgkg^{-1}) set by Chude *et al.* (2012). The mean P values differed significantly ($P < 0.05$) across the LUT and occurred in the increasing order: fallow (10.48mgkg^{-1}) < yam (29.79mgkg^{-1}) < cassava (35.49mgkg^{-1}) <vegetable (69.91mgkg^{-1}). These soil P values are well higher than those reported by Nkwopara *et al.* (2021) under selected LUT in Orlu area of the same humid tropical rainforest region of southeast Nigeria. The relatively high soil P levels recorded in the present study may be a function of the organic agricultural systems widely practiced in the studied area. Results of the exchangeable base cations (Ca, Mg, Na and K ions) showed the preponderance of Ca and Mg in the exchange site over Na and K ions which corroborates the findings of Ayolagha *et al.* (2012) and Egboka *et al.* (2021). Following the ratings of FDARL (1985), calcium and magnesium contents were moderate to high in the studied LUT while potassium and sodium ions ranged from low to very low amounts (Table 1). The effective cation exchange capacity (ECEC) of the soils varied significantly ($P < 0.05$) across the LUT with moderate mean values of 7.54, 8.70, 3.96 and 7.41cmolkg^{-1} in the cassava, vegetable, yam and fallow LUT, respectively (Table 1) while the percent base saturation (PBS) was very high in all the four LUT (Table 1) considering the critical value of 50% reported by Landon (1984). High to moderate levels of CEC and PBS in soils are usually necessary for good agricultural production.

Spore abundance of AMF in the studied land use types

Table 2 presents the results of AMF spore abundance in the studied LUT. A cumulative total of 712, 336, 380 and 480 spores of AMF were detected under the cassava, vegetable, yam and fallow land use types (LUT), respectively. Similarly, mean spore abundance of the AMF in the studied soils were 237, 112, 126 and 160 (spores 100g^{-1} soil) in the cassava, vegetable, yam and fallow LUT, respectively. Hence, soils of the cassava field harboured higher number of AMF spores compared to the three other LUT. The finding of highest mycorrhizal spore numbers in the cassava LUT may be linked to the report of Silveira *et al.* (2015) who stated that cassava is extremely well-adapted to acid-infertile soils with low P levels and is therefore, one of the crops most dependent on AMF associations. In general, the mean spore abundance (112 - 237 spores 100g^{-1} soil) recorded in the present study were higher than the ranges of 68.6 - 72.6 (spores 100g^{-1} soil) and 39 - 71 (spores 100g^{-1} soil) reported by Delvian (2021) and Egboka *et al.* (2022), respectively, but lower than the range of 307 - 1506 (spores 100g^{-1} soil) reported by Zerihum *et al*. (2013). Comparison of the mean spore abundance of AMF in the studied LUT showed that spore density recovered from the cassava field varied significantly ($P < 0.05$) higher than those obtained under each of the vegetable, yam and fallow LUT. Similarly, AMF spore abundance detected under the fallow field differed significantly ($P < 0.05$) higher than those recovered from each of the vegetable and yam LUT, but no significant difference was recorded between the vegetable and yam LUT. The cumulative spore density observed in this study which ranged from 336 AMF spores in the vegetable farm to 712 spores in the cassava farm compares with the cumulative spore numbers of 189 – 529 AMF spores reported by Dare *et al.* (2012) from soils of yam cropping systems in two agro-ecological zones of Nigeria. The finding of higher numbers of AMF spores in the cultivated cassava LUT than in the fallow field is consistent with that of Picone (2000), who inferred that disturbed ecosystem can trigger AMF sporulation because of grazing, disturbance and slow rate of decomposition than in natural ecosystems. Shi *et al*. (2007) also stated that the AMF is more likely to form spores when conditions prevail or host plant is stressed or disturbed. Generally, differences in AMF spore density may occur due to factors such as differences in land use system, edaphic cum climatic properties, vegetation type and cropping system, age of host plants and management practices as well as differential sporulation ability of AMF species (Muthukumar and Udaiyan, 2002; Husband *et al*., 2002).

Relationships between AMF spore abundance and selected soil properties of the study area

Correlations of AMF spore abundance with soil properties as presented in Table 3 showed a generally non significant ($P > 0.05$) effects apart from the result with organic carbon, where a significant positive effect ($r = 0.640^*$) occurred. Non-statistically significant correlations between soil properties and AMF spore abundance has also been documented in past studies (Jefwa et *al.* 2009; Delvian, 2021). Specifically, there were weak positive correlations between each of soil pH, total N and exchangeable K and AMF spore abundance, but negative correlations between spore abundance and each of available P and ECEC (Table 3). Jefwa *et al.* (2009) and Delvian (2021) also noted slight positive correlations between AMF spore abundance and each of organic C, total N and exchangeable K. Similarly, negative correlations of AMF spore abundance and available soil P were also reported by Isobe *et al*. (2007) and Egboka *et al*. (2022) whereas Mohammed *et al.* (2003) rather noted a negative relationship between the two. Positive correlations of soil properties with AMF spore abundance indicate the tendency of AMF spore numbers to increase with increasing levels of such soil properties while the negative correlations suggest a possible decrease in spore numbers as the soil properties increases.

Conclusion

The findings of this study revealed that differences in agricultural land use types (LUT) significantly influenced spore abundance of the arbuscular mycorrhizal fungi (AMF) in the humid tropical rainforest area of southeast Nigeria. Abundance of AMF spores in the selected LUT occurred in the decreasing order: cassava farm > fallow land > yam field > vegetable farm. There was a significant positive effect of organic carbon and slight evidence of positive effects of soil pH, N and K on AMF spore abundance, but negative correlations with soil P and ECEC. In the event of producing commercially-based AMF inoculum as biofertilizers in the studied area, rhizosphere soils of cassava fields should be considered as viable sources of soil-borne propagules of the AMF relative to other LUT investigated in the present study.

Table 1: Soil properties of the studied land use types

Land use type	pH	OC	TN	Av. P	TEA	Ca	Mg	K	Na	TEB	ECEC	BS
	H_2O	(g/kg)		(mg/kg)	(cmol/kg)							(%)
Cassava	5.77	6.23	0.77	35.49	0.73	4.43	1.58	0.29	0.52	6.81	7.54	90.23
Vegetable	5.53	14.06	1.19	69.91	0.56	4.89	1.92	0.74	0.59	8.14	8.70	93.32
Yam	5.9	8.36	1.04	29.79	0.38	2.17	1.02	0.05	0.35	3.58	3.96	87.00
Fallow	5.4	15.25	0.71	10.48	0.90	3.42	1.05	0.61	1.43	6.51	7.41	87.30
$LSD_{(0.05)}$	1.70	7.83	1.34	5.32	2.46	2.98	1.9	0.12	2.50	2.29	2.28	21.09

Data were reported as means of 3 replicate samples per land use type

O.C=Organic Carbon, TN=Total Nitrogen, TEA=Total exchangeable acidity, B.S=Base Saturation, Av. P=Available Phosphorus, C/N=Carbon-Nitrogen ratio, TEB=Total exchangeable bases, ECEC=Effective cation exchange capacity

Table 2: Spore abundance of the AMF in the studied land use types

Land use type	Total spore number (Cumulative)	Mean spore number ($100\ g^{-1}$ soil)
Cassava	712	237
Vegetable	336	112
Yam	380	126
Fallow	480	160
$LSD_{(0.05)}$		27

Total spore number and mean spore number represents the sum and average number of AMF spores, respectively, recovered per LUT

Table 3: Correlations between AMF spore abundance and some soil properties of the studied land use types

Soil properties	Correlation coefficients (r-values)
Soil pH	0.021
Organic Carbon (g kg^{-1})	0.640*
Total N (g kg^{-1})	0.306
Available P (mg kg^{-1})	-0.432
Exchangeable K (cmol kg^{-1})	0.235
ECEC (cmol kg^{-1})	-0.324

**** Significant at 0.05 probability level***

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