## **Research** Article



## Density of Arbuscular Mycorrhizal Fungi and Nutrient Status of Soils in Selected Land Use Types and Soil Depths

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**Abstract** | Arbuscular mycorrhizal fungi (AMF) are one of the most beneficial components of the soil biota whose abundance in soil varies with land use type, soil depth and location. The study investigated the density of the AMF and nutrient status of soils in selected land use types and soil depths. Soil samples were collected from some fallow, cassava and pineapple fields in Ibadan and Ikwuano areas of Nigeria at 0-15, 15-30 and 30-45 cm depths and analyzed in the Laboratory. Spore densities of AMF varied significantly (P > 0.05) between the fallow and cultivated (cassava and pineapple) land use types in both locations. Across the soil depths, however, AMF spore density decreased significantly with depth in Ibadan, with mean values of  $54\pm6$ ,  $45\pm3$  and  $39\pm5$  spores 100 g<sup>-1</sup> soil at the 0–15, 15–30 and 30–45 cm, respectively. In Ikwuano, there was no significant differences among means, and mean spore densities were more abundant at the 15–30 cm depth ( $67\pm2$  spores 100 g<sup>-1</sup> soil), followed concordantly by the 0–15 cm ( $66\pm4$  spores 100 g<sup>-1</sup> soil) and lowest at 30–45 cm depth ( $64\pm3$  spores 100 g<sup>-1</sup> soil). The status of soil nutrient elements (C, N, P, Ca, Mg, K and Na) were relatively higher in Ikwuano than in Ibadan soils. Spore density, essentially, correlated significantly positive (r = 0.910<sup>\*</sup>, P > 0.05) with the exchangeable K<sup>+</sup>, but correlated significantly negative (r = -0.834<sup>\*</sup>, P > 0.05) with total N in the fallow field. The density of the AMF was higher in the fallow than the cultivated land use types, and more at the 0–15 cm depth relative to the subsoil depths.

Received | May 31, 2021; Accepted | November 11, 2021; Published | March 31, 2022

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**Citation** | Egboka, N.T., O. Fagbola, U.N. Nkwopara, N.H. Okoli, A.I. Afangide and T.V. Nwosu. 2022. Density of arbuscular mycorrhizal fungi and nutrient status of soils in selected land use types and soil depths. *Sarhad Journal of Agriculture*, 38(2): 633-647. **DOI** | https://dx.doi.org/10.17582/journal.sja/2022/38.2.633.647

Keywords | Arbuscular mycorrhizal fungi, Spores, Soil nutrients, Ibadan, Ikwuano

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### Introduction

Interactions between soil microorganisms and plant roots at the soil-root interface result into various forms of associations (Barea *et al.*, 2002), which could be either beneficial or detrimental to the interacting species. Mycorrhiza is a symbiotic association between fungi and plants in which a fungus lives within or outside the roots of plants, forming a mutualistic relationship that is usually beneficial to both partners



(Tedersoo *et al.*, 2020). Arbuscular mycorhizal fungi (AMF) are one of the commonest and beneficial soil microbial communities in both agricultural and natural ecosystems (Leal *et al.*, 2009), which establish endomycorrhizal associations with over 85% of plant families (Smith and Read, 1997). In mycorrhizal association, the AMF protects the host plants against environmental stresses and enhances their uptake of inorganic minerals while the plants in turn, offer carbon compounds (photosynthates) to the AMF (Smith and Read, 2008). The association contributes to induced plant's resistance against pathogenic organisms in soil and tolerance to abiotic stresses such as drought (Smith and Read, 2008).

According to Kabir *et al.* (2003), soil fungi constitute a substantial part of the soil biomass having several vital roles in soil, including soil aggregation, organic matter decomposition, nutrient cycling and mycorrhizal symbioses. Symbiotic mycorrhizal fungi, in particular, form a major part of the microbes influencing plant growth and nutrient uptake (Johansson *et al.*, 2004). Relative to non-mycorrhizal plants, plants participating in mycorrhizal symbioses usually have an increased nutrient uptake (Smith *et al.*, 2010), greater tolerance to heavy metals toxicity (Rozpadek *et al.*, 2014) and higher resistance to drought and salinity (Auge, 2001).

In addition, the AMF also enhances the growth rate and survival of seedlings of many tropical plant species (Janos, 1980). They enhance plant's uptake of water and nutrients, especially phosphorus (P), and improves their ability to fix nitrogen, thereby enabling them to survive in the tropical marginal environments (Requena *et al.*, 2001). Mycorrhizal association also evidently enhances the uptake of micronutrients such as iron (Kim *et al.*, 2009), Zn (Ryan *et al.*, 2003) and Cu (Toler *et al.*, 2005) in plants, among others (Ryan *et al.*, 2004).

Traditionally, studies of the density and diversity of AMF has been based on the examination of relative abundances of the spores, which are distinguishable by their morphological characteristics (Muleta *et al.*, 2008; Sale *et al.*, 2015). This is mainly because the fungi involved in arbuscular mycorrhizal symbioses are obligate symbionts and reproduce essentially by soil-borne spores (Eun-Hwa *et al.*, 2013). Although molecular methods are now available for the assessment of AMF populations and diversity, spores con-

stitute one of the most important infective propagules of the AMF and they are vital in the isolation, quantification and identification of arbuscular mycorrhizal fungi (Smith and Read, 2008).

An understanding of the influence of land use systems and changes in land use types on the density of AMF is essential for harnessing the potentials of these important group of microbes in improving agricultural productivity, especially in impoverished soils. According to Soka and Ritchie (2015), studies of AMF populations and species diversity, and their roles in different land use types are vital for understanding the impact of land use changes on ecosystem functions. Many ecological studies of AMF indicate that the density and occurrence of AMF species decreases with intensification in land use (Oehl et al., 2003; Tchabi et al., 2008). Van-der-Heijden et al. (1998), had noted that developments in agricultural land use can change the whole range of AMF associations that are particularly suited to specific plants. Lower AMF species richness was reported in arable lands, while species-rich communities of AMF were observed under different perennial (forest) and natural ecosystems (Snoeck et al., 2010). Sanders et al. (1996) observed a variable response of different plant species to different species of AMF and a reduction in the abundance and diversity of indigenous AMF, particularly in disturbed arid and semi-arid environments, while Ndoye et al. (2012) noted a positive influence of land use systems (with Acasia plant species) on the diversity and spore abundance of AMF as well as on the functions of soil microbial communities.

The effect of depth on soil microbial populations, including the arbuscular mycorrhizal fungi, is also well known. Arbuscular mycorrhizal fungi are ubiquitous, occurring in virtually all climates and ecosystems (Barea et al., 1997) and at various depths of soil (Dalpe et al., 2000). Findings from independent researchers (Muleta et al., 2008; Oehl et al., 2005; Yang et al., 2010) indicate that AMF communities in subsoil layers differ from those of topsoils in terms of density, species diversity and community composition. In an arid habitat, Taniguchi et al. (2012), observed a decrease in AMF colonization with depth, which was maintained up to the 1 meter depth. In contrast, Gucwa-Przepióra et al. (2013) reported an increase in spore numbers of AMF and root colonization rate with depth, down to the 60 cm, in a heavy metal contaminated site.



While an appreciable number of research works are available on the density of AMF in specific land use types (Leal *et al.*, 2009; Grantina *et al.*, 2011; Ndoye *et al.*, 2012; Dare *et al.*, 2012; Zerihum *et al.*, 2013), only a few took into cognizance the effects of soil depth on the population density of the AMF (Yang *et al.*, 2010; Taniguchi *et al.*, 2012; Gucwa-Przepióra *et al.*, 2013). However, information on abundance of the AMF in relation to nutrient levels of soil is still scanty. This study therefore, investigated the density of arbuscular mycorrhizal fungi and nutrient status of soils in selected land use types and soil depths.

### Materials and Methods

### Description of study areas and sites

The study was conducted on soils of Ibadan (IB) in Oyo State and Ikwuano (IK) in Abia State, both in the southern hemisphere of Nigeria.

The study sites in Ibadan (IB) are located within the University of Ibadan Teaching and Research Farm in Ibadan North Local Government Area of Oyo State, Nigeria and lie between latitudes 7°48'34" and 7°28'41" N and longitudes 3°36'46" and 3°54'39" E of the equator, with elevations of 204.5 m and 193.4 m, respectively. The geology of the soils are basement complex rocks. The annual rainfall of the area is about 1200 mm with rainy season occurring between April and November. The temperature is generally high with the average annual minimum temperature being 21.9°C and the maximum is 32.5°C. The mean monthly temperature ranges between 24°C and 28°C. Humidity is high in the early hours of the day but sharply decreases in the afternoon. The mean value at 6 a.m is 92.98%, while it is 61.4% at 4.00 pm (Akinbola et al., 2014).

In Ikwuano (IK), the study sites which lie between latitudes 5°29'42" and 5°28'58" N and longitudes 7°34'29" and 7°33'28" E of the equator with the elevations of 273 m and 296 m, respectively, are both located in Umuokwor, Oboro community in Ikwuano Local Government Area of Abia State, Nigeria. The soils are of coastal plain sands origin. The area is characterized by rainforest vegetation of the southeast geopolitical region of Nigeria, and is typical of the degraded humid forest ecology of the sub-Saharan Africa (IITA, 1996). The rate of precipitation in the area is high (over 2,000 mm per annum) with the peaks occurring between August and September. The ranges of air temperatures and relative humidity are 21°C to 31°C and 42% to 80%, respectively (Chukwu, 2013).

### Soil samples collection and analysis

Two sites were sampled in each of the two locations (Ibadan and Ikwuano). Within each site, three land use types namely cassava, pineapple and fallow fields were identified. Guided by simple random sampling technique, 3 core soil samples were collected in each of the 3 land use types from 0–15, 15–30 and 30–45 cm depths using a soil auger. Thus a total of 9 core soil samples were collected per land use type. For each land use type in one location, samples collected from the same soil depth were bulked together to obtain composite samples of the respective soil depths. Soil samples were air dried at room temperature in preparation for laboratory analyses. Each composite sample was divided into two subsets: to determine the physical and chemical properties of the soils and for the estimation of AMF population.

Particle size distribution was determined by hydrometer method of Gee and Or (2002), Soil pH was determined in a 1:2.5 soil to liquid suspension (20 g soil and 50 ml distilled water) using the glass electrode pH meter (Hendershot et al., 1993), Soil organic carbon was estimated by the Walkley and Black wet oxidation method of Mclean (1982) and total nitrogen by the micro kjedahl method of Bremner as modified by Udo et al. (2009). Available phosphorus was determined calorimetrically by Mehlich III method (Mehlich, 1984) using UV spectrophotometer set at the wavelength of 882 nm, while exchangeable cations were extracted in Mehlich III solution and determined instrumentally by Atomic Absorption Spectrophotometry (AAS) method (Spark, 1996). Effective cation exchange capacity (ECEC) was calculated by the summation of the total exchangeable bases and exchangeable acidity.

### Extraction and enumeration of AMF spores

The population of arbuscular mycorrhizal fungi (AMF) spores in the soils was estimated using the wet sieving and decanting method as described by Gerdemann and Nilcoson (1963). A 100 g of each soil sample was mixed with a convenient volume of water in a large beaker (500 Ml) and stirred thoroughly with a glass rod to obtain a uniform suspension. The suspension was allowed to settle for 30 s and the supernatant was decanted through sieves of

Table 1: Soil properties of selected land use types in two locations of southern Nigeria.

	Ibadan			Ikwuano			
Soil property	Fallow	Cassava	Pineapple	Fallow	Cassava	Pineapple	
pH (H <sub>2</sub> O)	6.00 ±0.44	6.22±0.16	6.27±0.04	4.50±0.14	4.15±0.07	4.54±0.12	
Organic C (g kg <sup>-1</sup> )	11.67±1.35	9.24±2.18	10.92±2.38	17.57±5.49	20.30±4.51	14.56±2.53	
Total N (g kg <sup>-1</sup> )	1.67±0.19	2.59±0.41	1.91±0.46	1.87±0.43	2.38±0.20	2.19±0.13	
C/N	7.83±1.66	3.94±0.88	5.88±0.77	9.29±1.88	8.31±1.80	6.83±1.32	
Avail. P (mg kg <sup>-1</sup> )	15.50±0.29	16.17±0.24	18.17±0.28	29.67±0.25	21.83±0.23	13.00±2.17	
TEA (cmol kg <sup>-1</sup> )	5.33±2.61	5.71±2.62	5.21±4.32	5.68±8.97	5.92±6.46	6.05±0.25	
Ca <sup>2+</sup> (cmol kg <sup>-1</sup> )	1.33±0.10	1.45±0.19	$1.65 \pm 0.38$	3.99±1.60	5.51±2.05	1.75±0.66	
Mg <sup>2+</sup> (cmol kg <sup>-1</sup> )	$1.67 \pm 0.13$	1.73±0.11	1.52±0.38	1.92±0.42	1.96±0.30	1.42±0.16	
K <sup>+</sup> (cmol kg <sup>-1</sup> )	0.17±0.04	0.35±0.05	0.28±0.02	0.38±0.05	0.43±0.04	0.44±0.03	
Na <sup>+</sup> (cmol kg <sup>-1</sup> )	$1.00\pm0.02$	1.27±0.14	$1.02 \pm 0.07$	0.13±0.03	1.21±0.09	1.18±0.07	
TEB (cmol kg <sup>-1</sup> )	4.34±0.23	4.80±0.42	4.47±1.14	7.43±2.08	9.11±2.46	4.79±0.86	
ECEC (cmol kg <sup>-1</sup> )	9.68±0.42	10.51±0.50	9.67±1.63	13.11±1.98	15.02±2.36	10.84±0.81	
Sand (g kg <sup>-1</sup> )	864.83±16.31	824.00±15.66	884.67±18.77	840.00±30.11	845.00±28.13	841.00±28.04	
Silt (g kg <sup>-1</sup> )	65.56±12.21	61.83±10.71	28.50±13.73	41.33±14.35	35.00±12.03	32.33±10.25	
Clay (g kg <sup>-1</sup> )	129.67±7.01	114.17±9.93	86.83±6.88	121.00±15.42	120.00±17.65	126.67±19.42	

Data were reported as means ± standard errors. TEA = Total exchangeable acidity, TEB = Total exchangeable bases, ECEC = Effective cation exchange capacity

diameter 500, 212, 106 and 53 -  $\mu$ m, arranged in that sequence. The process was repeated three times for each sample. Particles in the 106 and 53- $\mu$ m mesh sizes were collected and centrifuged at 1800 rpm for 2 min. The sediment was resuspended in 40% sucrose solution and centrifuged again at 1800 rpm for 1.5 min to allow for flotation of spores. The spores in suspension were filtered and quantified by direct counting under a compound microscope using the X40 objective. The density of AMF spores in the soil was expressed as number of AMF spores in 100 g of soil.

#### Statistical analysis

Measured variables were analyzed using descriptive statistics with the aid of the GenStat discovery edition 4.0. Means were subjected to analysis of variance (ANOVA) to test for their statistical differences and significant means were separated using the Duncan's multiple range test. Relationships between AMF spore density and selected soil properties (nutrient parameters) were determined using the Pearson correlation analysis at 0.05 level of probability.

### **Results and Discussion**

Soil properties of three land use types in Ibadan and Ikwuano, southern Nigeria

The pH of Ibadan (IB) soils ranged from an average

of 6.00±0.44 in IB-fallow to 6.27±0.04 in IB-pineapple (Table 1). These range of pH (6.00 - 6.27) of soils of Ibadan area indicates slightly acidic soil reactions. Similarly, Ikwuano (IK) soil pH ranged from 4.15±0.07 in IK-Cassava to 4.54±0.12 in IK-Pineapple (Table 1), showing a very strong to strong acid reactions (Adebayo *et al.*, 2009).

The status of the soil nutrient elements (C, N, P, Ca, Mg, K and Na) were relatively lower in Ibadan soils in comparison to the soils of Ikwuano area (Table 1). Organic carbon occurred in low to moderate amounts  $(9.24\pm2.18 - 11.67\pm1.35 \text{ g kg}^{-1})$  in Ibadan soils, but in moderate to high amounts (14.56±2.53 – 20.30±4.51 g kg<sup>-1</sup>) in soils of Ikwuano area. This is with reference to Greg (2004) who placed the preferred values of organic carbon in soils at values above 20 g kg<sup>-1</sup> and not lower than 10 g kg<sup>-1</sup>. The concentrations of total nitrogen vary from medium to high amounts, ranging from 1.67±0.19 – 2.59±0.41 g kg<sup>-1</sup> in Ibadan and from 1.87±0.43 to 2.39±0.13 g kg<sup>-1</sup> in Ikwuano. In their ratings of fertility classes of Nigerian soils for fertilizer use and management practices, Chude et al. (2012) reported the ranges of 0.6–1.0, 1.1–1.5, 1.6– 2.0 and 2. –2.4 g kg<sup>-1</sup> as low, moderately low, medium and high, respectively, for total nitrogen. Specifically, organic carbon and total nitrogen contents in Ikwuano were highest at the cassava field (C =  $20.30 \pm 4.51$ 



g kg<sup>-1</sup>, N =  $2.38\pm0.20$  g kg<sup>-1</sup>) compared to the two other land use types. In Ibadan, however, total nitrogen was also highest at the cassava field (2.59±0.41 g kg<sup>-1</sup>), whereas the highest content of organic carbon  $(11.67 \pm 1.35 \text{ g kg}^{-1})$  occurred at the fallow field. The effect of fallow on organic matter build-up has been widely reported by different authors (Tian et al., 2005; Aguilera et al., 2013; Ahukaemere et al., 2020). The C:N ratio, an index of the degree of biological activities in soils was low in soils of both locations. This must have resulted from the very high levels of total N in the studied soils. According to Watson et al. (2002), nitrogen is more rapidly released into the soil at low C:N ratios. In general, a good balance of C:N ratio ranging from 25-35 is necessary to maintain microbial activity (Kutsanedzie et al., 2015). Results showed that, in both locations, the C:N ratio was highest at the fallow fields (IB-fallow =  $7.83 \pm 1.66$ , IK-fallow =  $9.29 \pm 1.88$ ) compared to those cultivated to cassava and pineapple (Table 1). This is in tandem with Fantaw-Yimer et al. (2007), who reported lower C:N ratios for arable soils relative to soils under forest field. However, the result disagrees with studies by Eyayu and Mamo (2018) who observed higher C:N ratio in cultivated land than forest land, and Abbasi et al. (2007) who noted lower ratios of carbon to nitrogen in the soils of natural vegetation than that of arable lands.

Values of available phosphorus was generally moderate (15.50±0.29 – 18.17±0.28 mg kg<sup>-1</sup>) in Ibadan soils, but moderate to high (13.0±2.17 to 26.6±0.25 mg kg<sup>-</sup> <sup>1</sup>) in soils of Ikwuano area. According to Chude et al. (2012), soil available P value is low at  $3-7 \text{ mg kg}^{-1}$ , moderate at 7-20 mg kg<sup>-1</sup> and high at >20 mg kg<sup>-1</sup>. The higher concentrations of available P in Ikwuano than Ibadan soils, may be a function of the relatively higher content of organic carbon, since most of the P available in soil derives from the soil organic matter pool. In Ikwuano, the concentrations of available P within the three land use types, occurred in the order of fallow > cassava > pineapple fields, whereas the reverse was the case in Ibadan (Table 1). This contrasting results of soil available P across the land use types between the studied locations could be attributed to the differences in environment (Cao et al., 2012; Blake et al., 2000), cropping systems (Ohno et al., 2005) and/or soil type (Zhang et al., 2009). The findings in the available P content of Ikwuano land uses, where the fallow field had higher P values relative to the cultivated land use types, tally with that of Eyayu

(2018), who observed significantly higher concentrations of available P in the forest soils of Ethiopia than in the cultivated land use types.

Considering the land use types in Ibadan, the highest value (10.51±0.50 cmol kg<sup>-1</sup>) and lowest value  $(9.67 \pm 1.63 \text{ cmol kg}^{-1})$  of the effective cation exchange capacity (ECEC) was detected under the cassava and pineapple land uses, respectively. A similar trend also occurred in Ikwuano, where the cassava and pineapple land uses had the highest and lowest ECEC values of 15.02±2.36 and 10.84±0.81 cmol kg<sup>-1</sup>, respectively. In general, the soil ECEC of both locations when placed side by side, was higher in Ikwuano than in Ibadan area across the three land use types. This can be attributed to the corresponding higher contents of organic carbon in the soils of Ikwuano area than that of Ibadan, in all the three land use types (Table 1). Although the colloidal particles (clay and humus) together constitute the seat of ion exchange in soils, the soil organic matter (SOM) particularly play a vital role in soil cation exchange reactions, since it offers more negatively charged surfaces relative to clay particles (Brady and Weil, 2002). Thus, as the organic matter content of soils increases, the cation exchange capacity (CEC) also increases.

# Soil properties of three soil depths in Ibadan and Ikwuano, southern Nigeria

The average range of pH of Ibadan (IB) soils was 6.13±0.11 in IB-15-30 cm depth to 6.33±0.08 in IB-0-15 cm; a range of pH classified also as slightly acidic soils. In Ikwuano (IK), the pH values ranged from 4.42±0.13 in IK-15-30 cm depth to 4.48±0.16 in IK-0-15 cm (Table 2), which equally qualify the Ikwuano soils as very strong acid to strong acid soils. The pH ranges in each of the locations which fall within the same classes of soil pH, irrespective of land use types and soil depths, reflects strong influence of the parent materials from which the soils were derived.

In Ikwuano, organic carbon and total nitrogen contents were highest at the 15–30 cm depth (C =  $18.06\pm4.33$ g kg<sup>-1</sup>, N =  $2.28\pm0.33$  g kg<sup>-1</sup>) in comparison with the two other soil depths (0-15 cm and 30-45 cm). Similarly, in Ibadan, the highest contents of organic carbon and total nitrogen were recorded at the 15-30 cm (11.62±1.79 g kg<sup>-1</sup>) and 30-45 cm (2.24±0.50 g kg<sup>-1</sup>) depths, respectively. These findings of higher concentrations of organic carbon and nitrogen contents in a subsoil depth than the topmost depth of soil

Table 2: Soil properties of three soil depths in two locations of southern Nigeria.

	Ibadan			Ikwuano			
Soil property	0 – 15cm	15 – 30cm	30 – 45 cm	0 – 15cm	15 – 30cm	30 – 45 cm	
pH (H <sub>2</sub> O)	6.33±0.08	6.13±0.11	6.24±0.75	4.48±0.16	4.42±0.13	4.29±0.11	
Organic C (g kg <sup>-1</sup> )	11.50±1.77	11.62±1.79	8.68±2.35	17.36±5.34	18.06±4.33	17.01±3.68	
Total N (g kg <sup>-1</sup> )	1.84±0.44	2.10±0.21	2.24±0.50	2.05±0.31	2.28±0.33	2.10±0.23	
C/N	7.18±1.54	5.62±0.79	4.74±1.40	8.97±2.30	7.71±1.19	7.75±1.48	
Avail. P (mg kg <sup>-1</sup> )	13.00±0.30	23.67±0.23	13.17±0.26	27.83±0.18	19.33±0.26	17.33±0.11	
TEA (cmol kg <sup>-1</sup> )	5.33±0.80	5.35±3.65	5.25±1.74	5.37±9.56	6.05±5.67	6.21±4.33	
Ca <sup>2+</sup> (cmol kg <sup>-1</sup> )	1.76±0.28	$1.40\pm0.14$	1.30±0.18	3.59±1.64	3.59±1.59	4.06±1.83	
Mg <sup>2+</sup> (cmol kg <sup>-1</sup> )	$1.70\pm0.07$	$1.60 \pm 0.14$	$1.49 \pm 0.17$	1.93±0.37	1.79±0.33	1.58±0.59	
K <sup>+</sup> (cmol kg <sup>-1</sup> )	0.33±0.03	0.27±0.02	0.32±0.06	0.47±0.04	0.38±0.04	0.38±0.04	
Na <sup>+</sup> (cmol kg <sup>-1</sup> )	1.11±0.02	$1.07 \pm 0.04$	1.18±0.15	1.21±0.20	1.17±0.06	1.14±2.15	
TEB (cmol kg <sup>-1</sup> )	4.91±0.77	4.35±0.30	4.30±0.51	7.20±2.07	6.94±2.02	7.17±2.02	
ECEC (cmol kg <sup>-1</sup> )	10.71±0.32	9.71±0.47	9.55±0.68	12.58±1.92	13.00±1.94	13.39±2.10	
Sand (g kg <sup>-1</sup> )	853.50±17.12	819.83±21.95	840.17±19.36	851.67±29.37	842.67±28.66	831.67±27.11	
Silt (g kg <sup>-1</sup> )	45.33±10.51	65.00±16.18	45.50±6.16	30.67±11.55	39.67±12.82	38.33±12.81	
Clay (g kg <sup>-1</sup> )	101.17±8.91	115.17±12.64	114.33±15.07	120.00±20.01	117.67±16.07	130.00±15.99	
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Data were reported as means  $\pm$  standard errors. TEA = Total exchangeable acidity, TEB = Total exchangeable bases, ECEC = Effective cation exchange capacity

occurred as a shift from that of Kunlanit et al. (2020), who reported the abundance of soil organic matter at the top 0–20 cm of the soil profile relative to the 20–100 cm depth. Eyayu and Mamo (2018) have also reported higher mean values of organic carbon and total N in the 0–20 cm depth of soil. The accumulation of organic matter in topsoil has been attributed to its position in the soil profile, which allows for direct input of organic litter (Sahrawat, 2004). Values of the C:N ratio were highest at the 0-15 cm depth in both locations compared to the subsoil depths (Table 2). This concurs with common knowledge as soil C:N ratio tends to decrease with soil depth.

In Ibadan, available P content was highest at the 15– 30 cm depth (23.67±0.23 mg kg<sup>-1</sup>) but lowest at the 0–15 cm (13.00±0.30 mg kg<sup>-1</sup>), whereas in Ikwuano, the concentrations of available P occurred in the order of 0-15 cm > 15-30 cm > 30-45 cm depths (Table 2). The effective cation exchange capacity (ECEC) of the soils decreased with soil depth in Ibadan (*i.e.* 0–15 cm > 15–30 cm > 30–45 cm), but in a reverse (increasing) order in Ikwuano (*i.e.* 0–15 cm < 15–30 cm < 30–45 cm). The ECEC results of Ikwuano soils where the deeper 30–45 cm depth had the highest value relative to the upper soil depths, contradicts the view of Brady and Weil (2002) that cations are mostly found abundant in the organic matter rich top-soils

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that are mixed with different organic materials at variable stages of decomposition which continuously release cations. However, the result in Ibadan, where ECEC was highest at the 0-15 cm depth, followed by the 15–30 cm and lowest at the 30–45 cm depth, conforms to expectations and is in tandem with the findings of Oladoye (2015) and Oyodele et al. (2008) who attributed decrease in ECEC values with depth to a corresponding decrease in organic matter levels. Density of arbuscular mycorrhizal fungi in three land use types at Ibadan and Ikwuano areas of southern Nigeria Soils of the fallow field in Ibadan (IB-fallow) had the highest density of AMF spores (54±7 spores 100 g<sup>-1</sup> soil) compared to soils of the cultivated land use types (cassava and pineapple fields) in the area (Table 3). In contrast, the highest spore numbers in Ikwuano was detected from soils cultivated to pineapple (71±2 spores 100 g<sup>-1</sup> soil), followed by the cassava land use type (68±2 spores 100 g<sup>-1</sup> soil) while the lowest AMF spore density (57±3 spores 100 g<sup>-1</sup> soil) was observed in soils of the fallow field (Table 3). Overall, soils of Ikwuano area, harboured higher numbers of AMF spores than soils of Ibadan area, in all the three land use types (Table 3). The results, therefore, showed a variation in spore numbers of the AMF with respect to both the land use types and locations, concurring with Dare et al. (2013) who stated that the population and composition of the AMF may be affected by



various factors which includes the land use or cropping systems practiced on the soil. Variations in spore density could arise as a result of the varying sporulation ability of AMF species under different land uses (Schenck and Kinloch, 1980), differences in agroecosystems and environmental conditions (Nandjui et al., 2013), or with differences in soil types (Marschner et al., 2001; Wieland et al., 2001). The higher spore densities observed in the Ikwuano land uses relative to those of Ibadan, irrespective of the high levels of acidity and available soil P in the Ikwuano area, contradicts a few studies (Gavito and Varela, 1995; Xavier and Germida, 1997; Redecker et al., 2013), who noted lower AMF spore densities with increased acidity and soil available P; but supports other similar works which reported positive influence of available P (Neumann and George, 2004; Subramanian et al., 2006; Muleta, 2007) and soil pH (Johnson et al., 1991; Mohammad et al., 2013; Tchabi et al., 2008) on the spore density of AMF.

Generally, the spore numbers of AMF detected across the three land use types in both locations, which ranged from 39±4 spores 100 g<sup>-1</sup> soil in IB-cassava to 71±2 spores 100 g<sup>-1</sup> soil in IK-pineapple (Table 3) vary from low to moderate when compared with the results of similar studies under different land use types. Zerihum et al. (2013) observed mean spore numbers (100 g<sup>-1</sup> soil) ranging from 307 to 1506 from acasia tree species in Ethiopia. In a tropical forest and pasture, Picone (2000) reported a range of 110 to 2600 spores 100 g<sup>-1</sup> soil, while Tao et al. (2004) noted 5 to 6400 spores 100 g<sup>-1</sup> soil under a valley savanna of the dry tropics. Dare et al. (2013) reported spore numbers ranging from 189 to 529 100 g<sup>-1</sup> soil from soils of yam cropping systems at four locations in Nigeria. However, in Northern Ethiopia, Birhane et al. (2010) detected low spore densities of 11 to 32 spores 100 g<sup>-1</sup> soil in dry deciduous woodlands under different acacia species.

Significant differences (P > 0.05) in spore density were observed between fallow and the cultivated (cassava and pineapple) land use types within each location. However, spore numbers of the cultivated land uses (cassava and pineapple fields) were not significantly different from one another in each of the locations (Table 3). Between the locations, there was no significant difference (P > 0.05) between spore densities obtained from IB-fallow (54±7 spores 100 g<sup>-1</sup> soil) and IK-fallow (57±3 spores 100 g<sup>-1</sup> soil). However, spore numbers obtained under IB-cassava (39±4 spores 100 g<sup>-1</sup> soil) and IK-cassava (68±2 spores 100  $g^{-1}$  soil) differed significantly (P > 0.05) from each other. Similarly, spore number obtained under pineapple land use in Ibadan (43±5 spores 100 g<sup>-1</sup> soil) was also significantly different from spore number detected under the same land use type (pineapple field) in Ikwuano (71±2 spores 100 g<sup>-1</sup> soil). The higher AMF spore density in the fallow field of Ibadan relative to those of the cassava and pineapple fields (cultivated land uses) is consistent with the report of Plenchette et al. (2005), who maintained that uncontrolled weeds (fallow fields) may positively influence the population and infectivity rate of the AMF. In intensive agricultural systems, the primary roles of mycorrhizosphere organisms may be marginalized, because microbial populations in conventional farming systems are easily altered by tillage operations and high use of mineral fertilizers and other agrochemicals (Gianinazzi et al., 2002). Again, the realization that the cultivated pineapple field harboured more spore numbers than the uncultivated fallow field in Ikwuano, corroborated the findings of Janos (1992) and Picone (2000) who reported that disturbed habitats induced the ability of AMF to sporulate due to grazing, disturbance and reduced decomposition rate than natural ecosystems. Similarly, Shi et al. (2007) noted that the sporulation of AMF is more likely to occur when the host plant is perturbed or stressed.

**Table 3:** Spore density of AMF in three land use types and soil depths at two locations of southern Nigeria.

Location		Mean spore number (100 g <sup>-1</sup> soil)	Soil depth	Mean spore number (100 g <sup>-1</sup> soil)
Ibadan	Fallow Cassava Pineapple	$39 \pm 4a$	0 – 15cm 15 – 30cm 30 – 45cm	45 ± 3ac
Ikwuano	Fallow Cassava Pineapple	68 ± 2c	0 – 15cm 15 – 30cm 30 – 45cm	67 ± 2b

Data were reported as means ± standard errors. Means followed by the same letters are not significantly different at 0.05 alpha level

Spore density of arbuscular mycorrhizal fungi at three soil depths in Ibadan and Ikwuano areas of southern Nigeria Across the soil depth in Ibadan, the ability of soil to support AMF populations decreased significantly (P > 0.05) with increasing soil depth, with mean values of  $54\pm6$ ,  $45\pm3$  and  $39\pm5$  spores of AMF 100 g<sup>-1</sup> soil at the 0–15, 15–30 and 30–45 cm depths, re-

spectively (Table 3). Similar results of a decrease in AMF spore density with increasing soil depth have been reported by Shukla et al. (2013) and Becerra et al. (2014). In Ikwuano, however, AMF spore density was highest at the 15-30 cm depth ( $67\pm2$  spores 100  $g^{-1}$  soil), followed concordantly by the 0–15 cm (66±4 spores 100 g<sup>-1</sup> soil) while the 30–45 cm depth also had the lowest number of AMF spores (64±3 spores 100 g<sup>-1</sup> soil). The findings in Ikwuano where the highest spore density was recovered from the middle 15-30 cm soil layer, corroborated that of Muleta et al. (2008) who observed a peak in spore numbers at the middle depth (20-30 cm) of a coffee plantation relative to the uppermost layer. Similarly, Gucwa-Przepióra et al. (2013) had reported an increase in spore density of AMF and root colonization rate to the depth of 60 cm, in a heavy metal contaminated site. In the contrary, the reduction in spore density of AMF with increasing soil depth, as was observed in Ibadan area, can be attributed to the fewer density of roots in lower depths of soil (Cuenca and Lovera, 2010). Other researchers had explained this on the basis of less organic carbon content (Oehl et al., 2005) and low levels of oxygen in deeper soil layers (Verma et al., 2010), since fungi are sensitive to low oxygen pressure which intensifies with depth (Brady and Weil, 2002).

Within the locations, spore numbers obtained from the three soil depths in Ikwuano area were not significantly different (P > 0.05) from one another (Table 3). However, in Ibadan, significant difference (P > 0.05) was observed between the 0–15 cm (54±6 spores 100 g<sup>-1</sup> soil) and 30–45 cm (39±5 spores 100 g<sup>-1</sup> soil) depths, with each of the two depths having no significant differences with the 15–30 cm depth (45±3 spores 100 g<sup>-1</sup> soil). Between the locations, there were significant differences (P > 0.05) in AMF populations obtained from each of the three soil depths (Table 3), indicating influence of the differences in soil type and environmental conditions.

Similar to the results of the land use types considered in this study, spore densities recovered from Ikwuano area also outweighed those of Ibadan soils in all the three soil depths. Even the highest spore density of  $54\pm 6$  spores  $100 \text{ g}^{-1}$  soil in Ibadan recovered from the 0-15 cm depth was less than the number realized from the least abundant depth (30–45 cm) in Ikwuano (with  $64\pm 3$  spores of AMF 100 g<sup>-1</sup> soil) (Table 3). In generally, the mean spore density obtained from the three soil depths of the present study, which ranged from  $39\pm5$  spores 100 g<sup>-1</sup> soil at IB-0–30 cm to  $67\pm2$  spores 100 g<sup>-1</sup> soil at IK-15–30 cm, were comparable with the numbers reported by Shukla *et al.* (2013) at four different depths of soil (0-10, 10-20, 20-30 and 30-40 cm) in Sagar, India; but lower than that of Becerra *et al.* (2014) who reported the range of 122 to 210 mean spores of AMF per 100 g soil at five soil depths (0-10, 10-20, 20-30, 30-40 and 40-50 cm) in saline soils of central Argentina.

## Arbuscular mycorrhizal fungi populations and soil nutrient levels

Land use types: Considering the land use types, there were both positive and negative correlations between AMF spore density and nutrient levels of soil in both locations.

In Ibadan, organic carbon and available phosphorus had a non significant (p > 0.05) positive correlations with spore density at the fallow and cassava land uses, but negative correlations at the pineapple field (Table 4), whereas total nitrogen correlated negatively with spore density in all the three land use types and this was significant at the fallow field ( $r = -0.834^*$ , p < 0.05). Many studies have reported negative correlations between spore numbers and soil properties, particularly with phosphorus (Kahiluoyo *et al.*, 2001; Emmanuel et al., 2010; Oehl et al., 2010; Dare et al., 2013; Nandjui et al., 2013). The negative relationships suggest a reduction in spore density of the AMF as levels of such soil properties increase in soil. However, in other similar studies, it was shown that soil parameters such as organic carbon (Tchabi, 2008; Hu et al., 2013), available P (Neumann and George, 2004; Subramanian et al., 2006; Muleta, 2007), and pH (Johnson et al., 1991; Mohammad et al., 2013; Tchabi et al., 2008), could affect AMF spore abundance positively. Muzakir (2011) observed increased AMF spore numbers and species diversity as the organic matter and soil pH increases. He thus, inferred that the amount and type of mycorrhizal spores was affected by the soil chemistry. With only a few exceptions, the exchangeable base cations showed positive correlations with spore density at the fallow field, but negative correlations at the cultivated land use types (cassava and pineapple fields). Specifically, there was a strong significant positive correlation with the exchangeable K<sup>+</sup> at the fallow field ( $r = 0.910^*$ , p < 0.05, Table 4).

In Ikwuano, however, there was a non significant positive correlation at the fallow field between spore



**Table 4:** Pearson correlation showing the relationships between AMF spore density and soil nutrients of three land use types in two locations of southern Nigeria.

Coefficient of correlation (r)							
	Ibadan			Ikwuano			
Soil nutrient element	Fallow	Cassava	Pineapple	Fallow	Cassava	Pineapple	
Organic carbon (g kg <sup>-1</sup> )	0.769	0.009	-0.082	0.710	0.043	-0.077	
Total Nitrogen (g kg <sup>-1</sup> )	-0.834*	-0.634	-0.045	0.640	-0.477	0.129	
Available P (mg kg <sup>-1</sup> )	0.116	0.059	-0.476	0.650	0.536	-0.280	
Exchangeable Ca <sup>2+</sup>	-0.266	-0.515	-0.765	0.714	0.029	-0.294	
Exchangeable Mg <sup>2+</sup>	0.236	0.131	-0.642	0.708	0.021	-0.207	
Exchangeable K <sup>+</sup>	$0.910^{*}$	-0.277	-0.555	0.615	0.445	0.334	
Exchangeable Na <sup>+</sup>	0.256	-0.409	0.121	0.720	0.153	-0.256	

\* Significant at 0.05 (5%) level of probability

**Table 5:** Pearson correlation showing the relationships between AMF spore density and nutrient levels of three soil depths in two locations of southern Nigeria.

Coefficient of correlation (r)							
		Ibadan		Ikwuano			
Soil nutrient element	0 – 15cm	15 – 30cm	30 – 45 cm	0 – 15cm	15 – 30cm	30 – 45 cm	
Organic carbon (g kg <sup>-1</sup> )	0.134	0.120	0.310	0.299	0.093	0.201	
Total Nitrogen (g kg <sup>-1</sup> )	-0.379	-0.351	-0.565	0.661	0.242	0.131	
Available P (mg kg <sup>-1</sup> )	0.080	-0.391	0.046	0.238	-0.121	-0.172	
Exchangeable Ca <sup>2+</sup>	-0.237	-0.542	-0.211	0.433	-0.107	-0.097	
Exchangeable Mg <sup>2+</sup>	0.138	-0.237	0.300	0.378	0.040	-0.015	
Exchangeable K <sup>+</sup>	0.756	0.234	-0.203	0.593	0.277	0.803	
Exchangeable Na <sup>+</sup>	0.065	-0.426	-0.283	0.046	0.246	-0.094	

density and each of the seven nutrient elements considered in this study (Table 4). A similar trend also occurred at the cassava land use, except in total N where correlation was rather negative, but also non significant (r = -0.477, p > 0.05). Positive correlations of spore numbers with nutrient elements suggest the tendency of the AMF spore to increase as the soil nutrient levels increases. However, over 98% of such results of positive correlation between AMF spores and soil nutrient elements from the results of the present study were non significant, stalling further inferences in that direction. Conversely, apart from the total N and exchangeable K<sup>+</sup>, all other nutrient elements evaluated in this study had a non significant negative correlation with spore density at the pineapple land use type (Table 4).

**Soil depths:** Across the soil depths in Ibadan, there was a non significant positive correlation between soil nutrients and spore density at the 0-15 cm depth, except in total N and exchangeable Ca<sup>2+</sup> where correlations were negative. At IB-15–30 cm, the result

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was in the contrast, as correlations were negative with the exception of organic C and exchangeable  $K^+$ , in which cases the relationships were rather positive. At IB-30–45 cm, however, organic C, available P and exchangeable Mg<sup>2+</sup> showed a non significant positive correlations with spore density whereas total N, exchangeable Ca<sup>2+</sup>, K<sup>+</sup> and Na<sup>+</sup>, all had non significant negative correlations with spore density (Table 5).

In Ikwuano, there was a non significant positive correlation at the 0–15 cm depth between spore density and each of the seven nutrient elements investigated in the present study (Table 5). These positive relationships tend to suggest an increase in AMF spore density as the soil nutrient levels increases. The result (of positive correlations) was also similar at the 15–30 cm depth, except in available P and exchangeable  $Ca^{2+}$  where correlations were rather negative (Table 5). Isobe *et al.* (2007) had also reported a negative correlation between soil available P and AMF spore density in upper soil layers. At the 30–45 cm depth, however, organic C, total N and exchangeable K<sup>+</sup> had



positive correlations with the spore density whereas the relationship was rather negative with the four other nutrient elements (Table 5).

Essentially, results of the correlation analysis between AMF spore populations and the soil nutrient levels at the three soil depths were all non-significant in both locations. This limits further inferences in the present study on the relationships between AMF spore density and soil nutrients across the soil depths. Similar inference was drawn by Shukla *et al.* (2013) who maintained that it is equivocal to establish direct cause and effect relationships between soil properties and the sporulation of AMF.

### **Conclusions and Recommendations**

Arbuscular mycorrhizal fungi (AMF) spores were more abundant at the fallow field relative to the cultivated (cassava and pineapple) land use types, and was higher at the upper 0-15 cm depth of soil compared to the subsoil (15-30 and 30-45 cm) depths. Although the findings of this research to a large extent, showed no definite pattern of relationship between AMF spore density and soil nutrients, significant (P < 0.05) positive and negative correlations were observed with exchangeable K<sup>+</sup> and total N, respectively, in the fallow land.

### Novelty Statement

The novelty of this research is to ascertain how soil nutrients affect the density of indigenous arbuscular mycorrhizal fungal communities in soil.

## Authors' Contribution

Nzube Thaddeus Egboka: Conducted the research and wrote the manuscript.

Olajire Fagbola: Supervised the research.

**Ugochukwu Nnamdi Nkwopara and Nnaemeka Henry Okoli:** Proofe read the manuscript and performed the statistical analyses, respectively.

Akaninyene Isaiah Afangide and Tochukwu Victor Nwosu: Helped in the Laboratory analyses and preparation of tables and figure.

## Conflict of interest

The authors have declared no conflict of interest

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