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ABUNDANCE AND DISTRIBUTION OF ARBUSCULAR MYCORRHIZAL FUNGI SPECIES IN LONG-TERM SOIL FERTILITY MANAGEMENT SYSTEMS IN NORTHERN NIGERIA

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□ *Soil fertility management systems (SFMS) can influence the community structure of arbuscular mycorrhizal fungi (AMF). Hence, long-term SFMS was studied. The SFMS comprised three legume combinations, urea application and solely maize as control. Spores were extracted by wet sieving, characterized and identified using their morphology. Interrelationships between cropping systems and occurrence of AMF species were analyzed with genotype by environment (GGE) biplot. Seventeen species were identified with Glomus species (47.05%) having highest value while Gigaspora species had the least (11.76%). Legume residues significantly ($P < 0.05$) increased spore population with the highest spore count (120 spores/100 g soil) obtained in plot under cowpea residue. Shannon Weiner index (H′) of maize/Lablab purpureus plot was highest (1.996) while that of sole maize system was the least (1.550). The GGE indicated Glomus intraradices as a stable species across all the SFMS. Community structure of AMF and function can be preserved using appropriate SFMS.*

Keywords: arbuscular mycorrhizal fungi, management systems, soil fertility

INTRODUCTION

The use of organic nutrient sources in soil management practices, especially the legume based cropping systems, is gaining ascendancy among farmers. The efficiency of nitrogen fixing legumes in crop productivity of such systems led to the development of low input systems, which reduced both energy and fertilizer inputs into agricultural lands (Vance, 2001). However,

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earlier reports showed that productivity in some legume-based cropping systems had been on the decline (Johnson et al., 1992). This has constituted a great problem in soil fertility management practices that are either solely or partially dependent on legumes. Leaving land to long fallow seemed to be the solution but as more agricultural lands are converted for other uses, fallowing becomes almost impossible in most countries. Thus it has become imperative to increase and improve productivity on the available lands, which are being subjected to continuous cropping. Therefore adoption of intensive cropping systems as a resolution is a necessity (Lyon and Peterson, 2005).

One of such intensive cropping systems commonly practiced by smallholder farmers is the cereal/legume rotation. This has been described as an effective system which has the potential to improve the productivity in nutrient deficient soils (Karlen et al., 1994). It is also generally accepted that the preceding legume crop provides nutritional supplements (usually nitrogen) for growth and development of subsequent crops. This has reduced the rate at which inorganic fertilizers are used in soil amendments. However, yield decline in cereal/legume rotation reported by Johnson et al. (1992) suggested that the actual mechanism involved in maximizing the effects of this cropping system is still poorly understood. Productivity in cropping systems may be influenced by many below-ground factors which interact to determine crop yield. These comprised of physical, chemical and biological components which are inter- and intradependent.

The microbial community is one of the biological components which are integral part of soil and crop productivity (Turco et al., 1990). Some have detrimental effects on crops while others are very important in nutrient acquisition and cycling. Mycorrhizal fungi have been described as soil micro-floras that are involved in nutrients transfer from soil to plants in natural ecosystems (Pate, 1994). Symbiotic associations with arbuscular mycorrhiza fungi (AMF) have been reported in many crops (Smith and Read, 1997). This group of fungi contributes to crop growth and development. Many research works have emphasized the importance of this symbiosis in P uptake, root growth, resistance to drought and soil borne pathogens (Galvez et al., 2001; Cooper, 1984; Osonubi et al., 1992; Jalali and Jalali, 1991). Also the development of efficient biological nitrogen systems (BNF) systems has been attributed to phosphorus (P) uptake enhanced by AMF (Ikombo et al., 1991). However, the efficiency of AMF colonization may depend on type of host, AMF species and ecotype (Mercy et al., 1990) which suggests that the activities of these groups of fungi may actually be a function of their community structure.

Soil fertility management practices, crop, and input systems have been reported as important factors influencing community structure and function of AMF. Vivikanendan and Fixen (1991) have shown the influence of rotation systems on the activities of AMF and reported a higher AMF colonization of maize following soybean than following barley. Kurle and Pfleger

(1994) also showed that AMF community responded to conversion from low to high input management systems. Furthermore, distribution and relative abundance of species within and among communities is an important factor that can increase our understanding on the activities of AMF in soil fertility management systems.

Therefore, in this study, the influence of organic and inorganic based soil fertility management systems on the community structure of AMF was examined.

MATERIALS AND METHODS

Soil samples were collected from selected plots in a long term field experiment located in Samaru, Northern Nigeria. This was established in 1997 for the Balanced Nutrient Management Systems (BNMS) program for West Africa to quantify maize yield in response to the immediate and residual effects of combining organic and inorganic sources of N. The soil class is Typic Ustropept and Eutric Cambisol (FAO/UNESCO, 1974). The experimental site was a gentle slope ($<5\%$) with sandy loam soil. The experimental treatments were divided into three sets. Set 1 comprised of four cropping systems: maize/*Centrosema pascuorum* (MCP), maize/*Lablab purpureus* (MLP), maize/cowpea (MC), and solely maize (M). Treatment in this set was incorporation of organic residues produced within each system. The legume crops were relayed in maize and their residues left on the plots. In set 2, treatment was application of urea nitrogen at rates 0 and 45 kg ha^{-1}. In Set 3, cow dung and poultry manure produced outside the system were used as organic residues supplemented with Urea N applied also at 0 and 45 kg ha^{-1}. In sets 2 and 3, P and potassium (K) were added yearly as triple super phosphate (TSP) and potassium chloride (KCl) at 10 and 30 kg ha^{-1} respectively. Soil samples for AMF spore count and identification were collected at maize planting in June 2005 and 2006 respectively. Each plot was randomly sampled at 0–15cm depth in eight locations using 30 cm soil auger to form composite sample. AMF fungi were isolated from three 100g soil sample by wet sieving through a series of 710, 500, 250, and 45 μm sieves (Brundrett et al., 1994). Spore abundance was determined by direct counting under a dissecting microscope. Species identification was based on morphological characteristics of the spores such as the size, color, shape, cell wall layering, type and mode of hyphal attachment. Spores were characterized by mounting in polyvinynl-alcohol-lactic acid-glycerin (PVLG) with Melzer's reagent (1:1 v/v) and broken to various degrees by pressing the cover slips on the slides (Brundrett et al., 1994). Spore characteristics were compared with species identity on the website of International Collection Centre for AMF (INVAM). The AMF species diversity was measured using the Shannon Weiner's index (H'), species evenness (E) (Franke et al., 2001) and

Simpson's index (*D*) (Hendrix et al., 1995). The ecological measurements were calculated from:

$$H' = -\sum Pi \ln Pi$$

$$D = \sum (ni/N)^2$$

$$E = H'/\ln X$$

Where *Pi* is the proportion of individuals in a species, *ni* is the number of each individual species, *N* is the total number of individuals and X is the number of species recorded. Spore counts were transformed by $\sqrt{}$(X+0.5). Analysis of variance (ANOVA) was performed using SAS mixed model systems (SAS, SAS Institute, Cary, NC, USA) and means compared using LSD. Interaction between the cropping system and species composition of AMF community were analyzed using the genotype by environment GGE Complete Biplot Analysis System Version 4.1 (CRC, Boca Raton, FL, USA). It is a system of analysis whereby genotypes can be grouped according to their stability in different environments assayed. There must be at least two environments and genotypes each, before the analysis can be conducted

RESULTS

Spore Populations of AMF in Organic and Inorganic Based Systems

The effects of cropping systems, crop residue and fertilizer application on the abundance of AMF were significant ($P < 0.05$). In set 1, application of legume residue significantly ($P < 0.05$) increased the AMF spore count compared to sole maize crop. Solely maize with 20 spores /100g soil was the least while *Lablab* had 300% increase in spore count compared to solely maize (Figure 1). Although the *Centrosema* system had a slight increase in spore count compared to that of *Lablab*, the difference was not significant ($P < 0.05$). Spore count in the cowpea system was significantly ($P < 0.05$) increased (500%) compared to that of solely maize and *Centrosema* (100%). Hence, legumes in cropping systems are an important factor which greatly influenced the abundance of AMF spores. In maize/*Centrosema* system, application of urea at 45 kg N ha^{-1} significantly ($P < 0.05$) reduced spore populations from 92 observed at 0 kg N ha^{-1} N to 66 per 100 g of soil sample(Figure 2). However, in maize/*Lablab* and maize/cowpea plots, spore counts increased with application of urea N. Urea application did not affect spore population on the sole maize plot; spore counts at the two N levels were similar. Set 3 systems further showed increase in spore population with organic residues (Figure 3). There was a significant ($P < 0.05$) increase

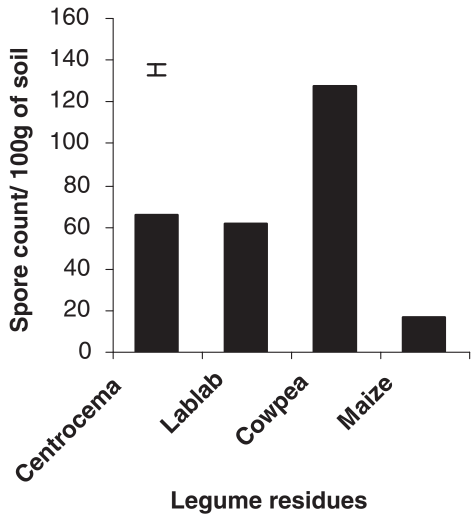

FIGURE 1 Effects of application of *Centrosema pasuorum*, *Lablab purpureus*, and cowpea residues on spore populations of AMF with solely maize as control. Bar represents standard error of deviation.

in spore population on plots without urea N in both poultry and cowdung systems. However differences occurred in the contribution of each organic residue. Spore population with cow dung was significantly ($P < 0.05$) higher than with poultry residue.

Distribution of AMF Species

A total of 17 species were identified from all the cropping systems (Table 1). The species were grouped into four genera of which the genus

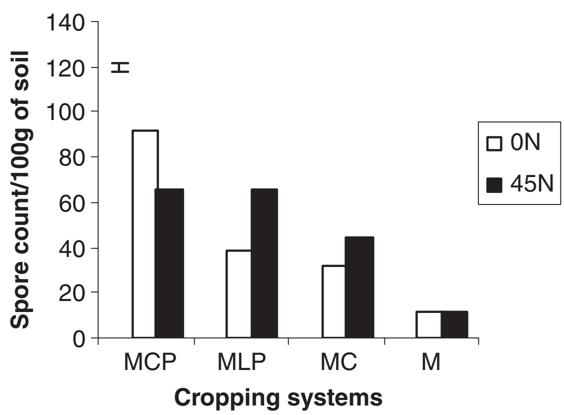

FIGURE 2 Spore populations of AMF in four cropping systems supplemented annually with urea N and/or plant residues obtained within the systems (set2). MCP = Maize/*Centrosema pascuorum*; MLP = Maize/*Lablab purpureus*; MC = Maize/cowpea and M = solely maize. Bar represents standard error of deviation.

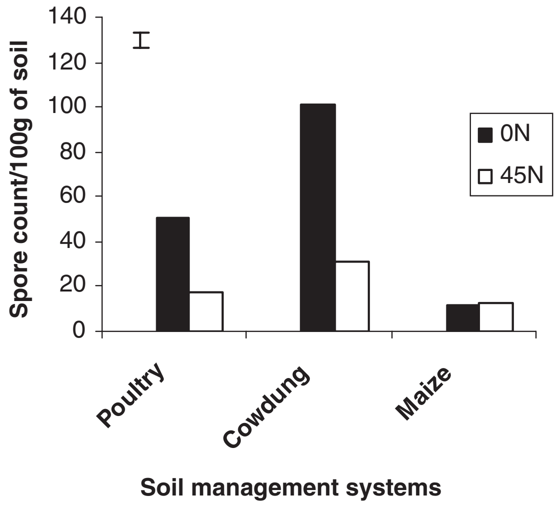

FIGURE 3 Population of AMF spores in long term experiments supplemented annually with urea N and/or animal residues (set 3). Bar represents standard error of deviation.

Glomus accounted for approximately 47% of the total spores isolated from all the systems (% occurrence was based on number of individual genus observed). *Scutellospora* accounted for 24% while *Acaulospora* and *Gigaspora* were 18% and 12% respectively. Also, *Glomus* was the most frequently occurring

TABLE 1 Percentage occurrence of AMF species per 100g of soil under different cropping systems

	% occurrence			
Species	MCP	MLP	MC	M
Glomus ambiosporum	4.15	1.66	2.79	6.19
Glomus clarum	16.81	15.35	15.55	15.93
Glomus etunicatum	2.32	4.98	2.54	7.08
Glomus geosporum	0.97	0.21	0.34	0.00
Glomus intraradices	22.22	21.16	25.70	25.66
Glomus lamellosum	1.16	0.21	0.51	0.00
Glomus mossae	0.97	1.87	0.17	0.00
Glomus multicaule	1.16	0.00	0.51	1.77
Acaulospora delicate	5.22	3.53	0.68	0.88
Acaulospora denticulata	1.55	8.51	0.17	5.31
Acaulospora elegans	0.00	0.62	0.34	1.77
Scutellospora calospora	9.08	4.98	2.87	12.39
Scutellospora dipurpurescens	9.47	5.19	1.01	6.19
Scutellospora heterogama	2.32	3.53	7.78	2.65
Scutellospora rubra	5.60	8.51	16.91	3.54
Gigaspora gigantean	16.23	15.77	9.81	9.73
Gigaspora rosae	0.77	3.94	12.34	0.88

Maize/*Centrosema pascuorum* (MCP), maize/*Lablab purpureus* (MLP), and maize/cowpea (MC) and solely maize (M) systems.

TABLE 2 Ecological measures of diversity for AMF propagules under different cropping systems

Cropping systems	H′	D	E
MCP	1.938	0.576	0.684
MLP	1.996	0.625	0.705
MC	1.792	0.814	0.633
M	1.550	0.138	0.547
SED	0.031	0.051	0.048

Shannon Weiner (H′), Simpson's indices (D) and species evenness (E).

Maize/*Centrosema pascuorum* (MCP), maize/*Lablab purpureus* (MLP), and maize/cowpea (MC) and solely maize (M) systems.

species within each system. In all the cropping systems, *Glomus intraradices* was the most frequently occurring AMF species, followed closely by *Glomus clarum* which had the most frequent occurrence under MCP and solely maize. *Gigaspora gigantea* and *Scuttelospora rubra* had the highest occurrence under MLP and MC respectively. Others with occurrence greater than 10% were *Scuttelospora calospora* under solely maize (12.39%). It is interesting to note that though genus *Glomus* had the highest percentage occurrence, yet, *G. geosporum, G. lamellosum* and *G. mossae* had 0% occurrence under sole maize system, while *G. multicaule* also had 0% under MLP system. All other species except *Acaulospora elegans* under MCP were represented under all the cropping systems.

Shannon Weiner's indices (Table 2) indicated diversity which was lowest in the solely maize system. The distribution of AMF species was also less even in the solely maize system as a result of absence of more than one species. H' value obtained for the maize/cowpea system was also low compared to other legume systems as a result of dominance of some species shown by higher D and a lower E values in this system. However, distribution of species in the maize/*Centrosema pascuorum* and maize/*Lablab purpureus* systems was similar. A more detailed analysis was shown by the biplot of PC1 and PC2 based on the abundance of each species. The principal component analysis explained approximately 95% of the interaction between the cropping systems and the community structure of AMF.

The length of the vectors in the biplot analysis indicated standard deviation in terms of population of individual species (Figure 4a). The longer the vector, the more informative, or discriminating the system. Therefore the maize/cowpea system showed the most varying number of each species and provided more information than the sole maize and other systems. The angle between the vectors is an indication of the correlation between the systems. All the angles were <90°; therefore the systems were all correlated. Maize/*Lablab* system had the smallest angle to the average environment axis which showed that it is more representative of a system that would support sporulation of the most frequent species. However similar results were

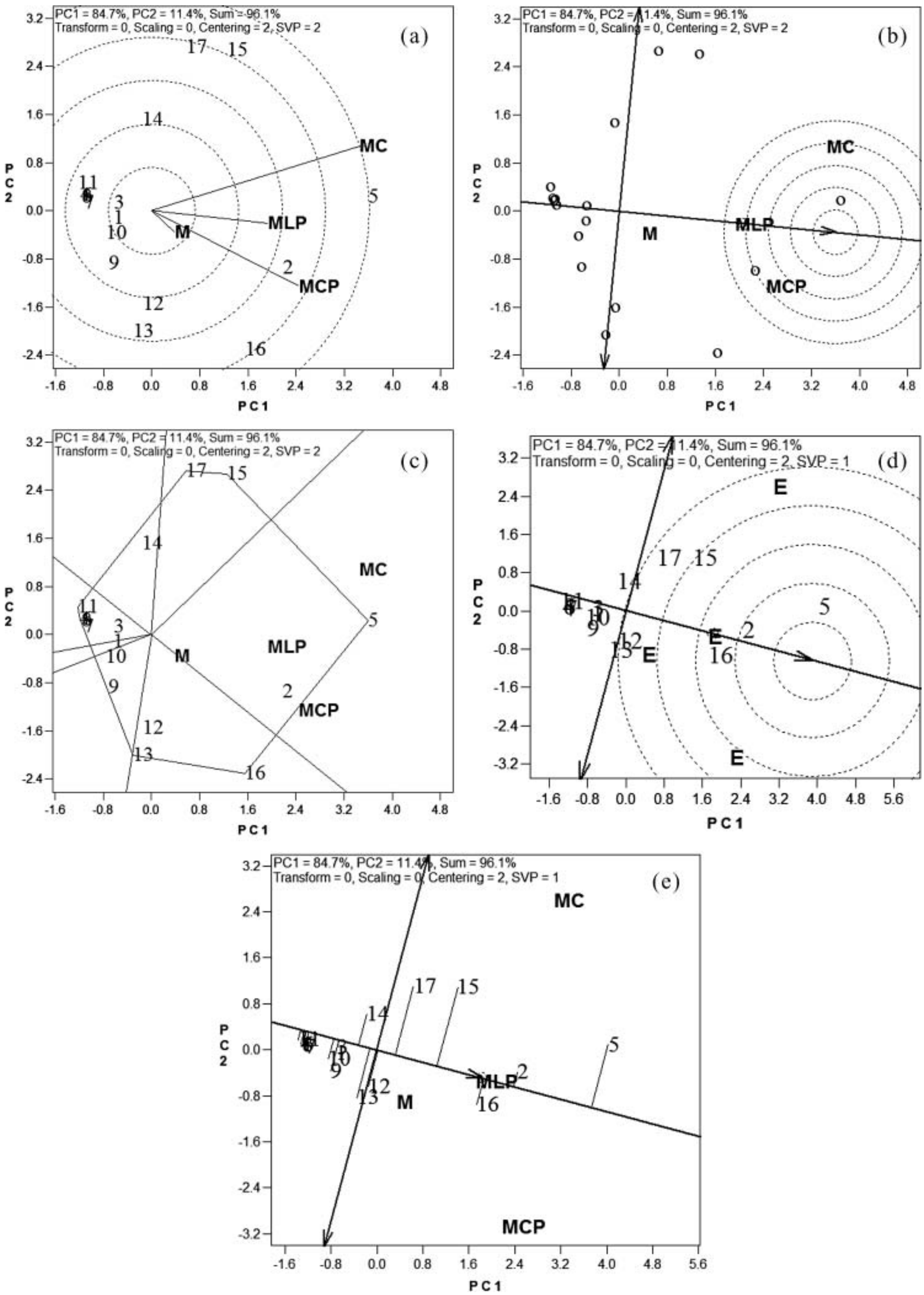

FIGURE 4 a) Relationship among four cropping systems based on the abundance of AMF species; b) Ranking the cropping systems based on their discriminating ability and representativeness; c) Occurrence of AMF species relative to the management systems; d) Ranking the component species of AMF with reference to mean population and stability across the systems; the average environment coordination view showing the mean population of individual species across the systems. Numbers in the figures represents species identity as listed in Table 1. Maize/*Centrosema pascuorum* (MCP), maize/*Lablab purpureus* (MLP), and maize/cowpea (MC) and solely maize (M) systems. PC1 and PC2 = Principal components 1 and 2 respectively.

obtained in other legume systems as shown by ranking the cropping systems based on their discrimination and representativeness (Figure 4b). Also, all the legume systems were grouped together in the same sector (Figure 4c) which indicated that the frequently occurring species were the same in the three legume systems. *Glomus intraradices* (5) occurred more in the legume systems which were distinctly different from the sole maize where *Gigaspora gigantea* (16) and *Scutellospora dipurpurescence* (13) were the most frequent species (Figure 4d). The average environment coordination view of the GGE biplot showed that *Gigaspora gigantea* and *Glomus clarum* were more stable across the cropping systems than *Glomus intraradices* though the latter had the highest mean population (Figure 4e).

DISCUSSION

The general objective of this study was to examine the influence of organic and inorganic based soil fertility management systems on the community structure of AMF. Several studies have shown the activities of these symbiotic groups of fungi in plant growth and development. However, an understanding of AMF species composition and distribution is an added advantage when studying their efficiency in soil and crop productivity.

The populations of AM spores varied in the four cropping systems. The differences in spore populations can be attributed largely to the effects of management system. Schenck and Kinloch (1980) had earlier reported differences in ability of AMF species to sporulate in different cropping systems.

Residue application in soil management systems is an important factor which may enhance AMF spore abundance (Borie et al., 2002). Types of residues were significant ($P < 0.05$) in set 1 and also relevant in sets 2 and 3 when compared with application of inorganic fertilizers. This might be an indication of variation in the quality and quantity of soil organic matter supplied by each residue as a result of differences in the rate of decomposition and nitrogen mineralization (Handayanto et al., 1995), which may influence spore germination, hyphal development and sporulation of AMF (Hepper and Warner, 1983). Legumes are very important crops which are incorporated into management systems not only for their residues but most importantly for their efficiency in fixing nitrogen into the soil through biological nitrogen fixation (BNF). Also Ahiabor et al. (2007) recently reported differences in the abundance of AMF spores in four legume systems, although spore abundance did not influence AMF infectivity.

The development of efficient BNF systems has been shown to be facilitated by mycorrhizal symbioses which enhances phosphorus uptake for development of the bacteria symbiont (Ikombo et al., 1991). Therefore in the legume systems, sporulation may be encouraged by hyphal development in response to nitrogen and phosphorus needs of the legumes. However, earlier reports by Egerton-Warburton and Allen (2000) showed that nitrogen enrichment reduced spore population and affected the community structure of AMF. Our results showed that application of urea may either enhance or limit AMF spore population in legume based systems. Therefore, we suggest that spore abundance could be an indication of the amount of residual N incorporated back into each system which was not encouraged by application of urea. The results also suggest that animal residues may be important in establishment and maintenance of AMF symbiosis in cropping system. However influence of each residue also differs greatly which may be attributed to variation in nutrient content of the manures (Yagodin, 1984).

Glomus species dominated the spore population obtained from all the systems as was reported in other studies (Hendrix et al., 1995; Sanginga et al., 1999.). The reason for this is not clear although it may be attributed to the promiscuity of *Glomus* species or the selective effects of maize which was continuously cropped over the years. The populations of some individual species were reduced in the sole maize compared to other systems. We are of the opinion that these species might have existed in greater populations in the past but reduced gradually as the effects of long-term continuous monocropping sets in. Also the absence of more species in the solely maize system could be an indication of gradual removal of some species from the AMF community as a defect of long term management system used in this case. However, it is difficult to draw a conclusion due to lack of information on the AMF status of the soil in the previous years as well as non-availability of similar sites without history of cultivation.

The most frequent species among all legume based systems were similar, however, this differs for maize-only systems suggesting that the influence of the legumes incorporated into the management systems was similar. *Glomus intraradices* and *Glomus clarum* may be selected as ideal species which would sporulate under soil conditions as dictated by the four management systems. This selection is based on the mean performance of *Glomus intraradices* and the stability of the latter as suggested by Yan and Kang (2003). This may be an indication that the test crop (maize) may particularly be selective for this species of AMF. Although several studies have indicated no host specificity in mycorrhizal symbiosis, however, there is increasing evidence that host plant selects for particular species of AMF. This report is in accordance to that of Johnson et al. (1992), where remarkably different AMF communities were observed in plots with continuous corn and continuous soybean. They therefore suggested that some degree of specificity may exist between hosts and AMF species.

This research work has therefore indicated that indigenous AMF community could be managed alongside soil fertility practices. We suggest that appropriate organic based systems could be used to preserve the existing structure, gradually restore the lost species and/ or introduce new species in soil.

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