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THE ECOLOGY OF ARBUSCULAR-MYCORRHIZAL FUNGI (AMF) UNDER DIFFERENT CROPPING REGIMES

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Abstract

The ecology of Arbuscular Mycorrhizal Fungi (AMF) in mono-cropping and low-input ideal agroforestry cropping systems of *Avena sativa* has been studied. Soil chemical heterogeneity, seasonality and nature of cropping system showed significant attributes on AMF. AMF percentage in roots and spore populations in soil were elevated in dry season compared to wet season. With respect to cropping regimes, mono-cropping systems exhibited highest root infection whereas the agroforestry systems possessed highest AM fungal spore populations. Generally, farming systems tested here possessed significant colonization of AMF, however, overall extent of colonization and spore densities were low. While assessing the correlation between soil chemical composition and AMF, electrical conductivity, organic carbon content, available potassium and saturation percentage showed a negative correlation. However, pH showed a positive correlation and available phosphorus content showed no correlation with AMF. Present study was aimed to view the importance of agroforestry in modern agriculture and normal agricultural system and the benefits associated with AM fungi.

Key words: AM fungi, Mono-cropping/agroforestry, *Avena sativa,* Ideal cropping system.

Introduction

Pakistan is one of the countries having highest population growth rate resulting an increased demand of land use and crop production. The traditional farming practices used in past have now been replaced by chemical practices because of the absence of fertile land (Tzouvelekas *et al*., 2001). The use of chemical fertilizers except from conventional methods is expensive and is not eco-friendly. It declines soil richness and environment quality as well (Parks & Seaton, 1996). The ability of the crops to resist plant pathogens is mainly tied to biological properties of soils. Soils with active soil biota exhibits good soil fertility because soil micro-organisms recycle soil nutrients and enhance soil strength (Altieri & Nicholls, 2003). The application of chemical fertilizers can cause nutrient discrepancies, low pest resistance and is a short term solution because it requires continuous input. On the other hand, the microbial biota of soil is a long-term investment in sustainable cropping systems and have unintended impact on the soil quality. The use of microbes as bio-fertilizers is ensured by continuous replacement of organic inputs resulting in healthy agriculture. A vast array of soil microbes are known for years which play vibrant role in nutrient cycling, uptake and maintenance of beneficial growing conditions. One of these soil micro-organisms is a fungi belonging to Phylum *Glomeromycota,* known as Arbuscular-Mycorrhizal Fungi (Schüßler *et al*., 2001). The AMF exists mutually in plant roots and are vital to soil processes (Bull, 1996). Root colonization by AMF considerably improves the host plant growth by increased nutrient uptake and greater resistance to plant pathogens and unfavorable ecological conditions.

Another promising eco-friendly biological farming practice is agroforestry systems due to its potential to improve soil fertility and microbial diversity (Young, 1997). Trees have long-term impacts on agriculture because of greater biomass accumulation and widespread root systems. These agroforestry systems ensures sustainable food production in developing tropical areas like Pakistan because they are more productive along with a greater diversity of microbial species and large arbuscular mycorrhizal consortium than mono-cropping ecosystems (Sanchez *et al*., 1996).The aim to evaluate AMF symbiosis in tree-based intercropping systems is to investigate the arbuscular mycorrhizal functioning and diversity in a system that approaches the management ideal for low input sustainable agriculture. There is great potential for the improvement of soil chemical and physical properties by agroforestry cropping systems having extra room of improvement in association with AM fungi. Agroforestry systems can provide absolute productivity, if conditions for the survival of AMF symbiosis are favorable.

The potential of AM fungi in plant growth is very clear; the purpose of the study was to inaugurate the diversity and status of Arbuscular-Mycorrhizal fungi in the most ideal and sustainable cropping system sand deviation in AMF diversity and distribution in response to seasonality and varying soil conditions. A utilization strategy can be developed by determining the mycorrhizal species that thrives best under different cropping systems and their dynamic relationship with crops and agroforestry trees.

Materials and Method

The study was under taken during 2012-2013 in Govt. Agricultural Research Farm, Bahawalpur, Pakistan. The agricultural farms in Bahawalpur were established about 50 years back and have the history of low fertilizer in puts. We selected 20 plots of one acre each for the present study from the agriculture farms and each plot was separated from the other by 1.5 meter wide walkway. During experimental year, the fields under study were cleared from weeds manually and 5 of each four cropping systems, i.e. *Eugenia jambolana*/Oat intercrop, *Mangifera*

indica / Oat intercrop, *Ficus benghalensis*/ Oat intercrop and Oat monocrop system) were selected. The sampling was done by complete randomized design two times a year (wet and dry seasons), with a four factorial treatment arrangement (4 cropping systems), from each plot (5 plots), replicated three times to avoid experimental error (2x4x5x3= 120 samples). Soil and root samples were collected during February and July to investigate the diversity and status of Arbuscular-Mycorrhizal fungi. Soil (5.0 Kg) was collected at each sampling point at different depths and was mixed to make a composite sample. Root samples were also collected along with soil at each site. Root and soil samples were placed in polythene bags, labeled and transported to the laboratory. After arrival, root samples were immediately washed, preserved in FAA (Formaldehyde-Acetic Acid-Alcohol, 5:5:90 ml) and refrigerated at 4^{o}C. Soil samples were sieved; air dried and used for the estimation of physical and chemical properties and AMF spore densities.

To assess the colonization status, the roots were cleared and stained according to the Phillips & Hayman (1970) protocol and modified by (Chaudhry *et al*., 2012). The infection percentage was measured by Biermann & Lindermann (1981) method.

300 grams of each soil sample was deposited to Soil and Water Testing Laboratories for Research, Bahawalpur, for the estimation of their physical and chemical characteristics. All the soil samples were clayey loam. The chemical properties of soil sampled in both wet and dry season are given in Table 1. AMF spores were extracted from rhizospheric soils by using the wet sieving and decanting technique of Gerdemann & Nicolson (1963). 15-20 spores of each isolated AMF morphotype were picked with tooth pick under a stereoscope and were mounted in PVLG (polyvinyl lactoglycerol) and Meltzer's reagent separately on the same slide under different coverslips. A slight pressure was applied to exclude extra mounting media and to clarify the spore structures by breaking their walls. All the spores were examined using Labomed Digi-2 Compound microscope. Identification manual of Schenck & Perez (1990) and International collection of Arbuscular-Mycorrhizal fungi (http://invam.caf.wvu.edu) were used to determine the AMF morphotypes. Spore densities were estimated as the number of spores per hundred gram of soil and values were subjected to diversity indices and frequency of occurrence.

The descriptive statistics of AMF colonization status and distribution was performed by Mini Tab 13. Pearson's correlation among soil physico-chemistry and AMF was assessed using SPSS 17.0. Analysis of variance (ANOVA) and Least Significant Difference among four treatment's colonization status was performed by using MSTATC. Shannon-Weiner diversity index of AMF species diversity was assessed manually.

Results

Average temperature and humidity of wet and dry seasons are given in Fig. 1. As displayed during wet season the average temperature during the day was 25.29^{o}C, however, it reaches up to 39.89^{o}C in dry months of the year. In contrast, average humidity was highest in wet season (i.e. 62.01%) and decreased to about half (i.e. 32.96%) in dry season. This change in climate resulted significant changes in colonization percentage of Arbuscular-mycorrhizal fungi in plant roots. Fig. 2 displayed the percentage of different mycorrhizal structures in plant roots. Hyphal percentage was highest in wet season but reduced in dry season. However, vesicular percentage remained almost same in both the seasons. Arbuscular colonization status and percentage of intra-radical spores was high in dry season but reduced significantly in wet season respectively. This might be due to more AMF colonization and distribution in stressed dry climatic conditions.

Along with seasonal variation AMF colonization was also varied among different cropping systems. Fig. 3 displays the AMF colonization status among different cropping regimes and the least significant difference (α=0.05) among them. Oat monocrop showed highest hyphal colonization percentage followed by *Ficus benghalensis*/ Oat intercrop, *Mangifera indica* / Oat intercrop and *Eugenia jambolana* / Oat intercrop system respectively. Vesicular and arbuscular percentage in four cropping systems followed similar pattern. Both parameters were highest in *Ficus benghalensis*/ Oat intercrop followed by Oat monocrop and *Mangifera indica* / Oat intercrop system. All these three systems lied in the same LSD category A except *Eugenia jambolana* / Oat intercrop with considerably low percentage of vesicles and arbuscules respectively. The percentage of intra-radical spores was generally low in all cropping systems. Maximum percentage was attained by Oat monocrop followed by *Mangifera indica* / Oat intercrop, *Ficus benghalensis*/ Oat intercrop (9.83%) and *Eugenia jambolana* / Oat intercropping site respectively.

Table 1. Physical and chemical composition of rhizospheric soil sampled from different cropping systems in wet and dry season.

Season	Cropping system	EC (dSm^{-1})	pH	OC (%)	P (ppm)	K (ppm)	SP (%)
Wet season	Oat Monocrop	8.34±1.47	8.06±0.07	0.76±0.05	6.04±3.33	162.4±15.4	49.2±1.02
	Mangifera indica / Oat intercrop	2.52±0.17	7.92±0.04	0.73±0.03	3.06±1.66	200.2±25.9	49.2±1.20
	Ficus benghalensis/ Oat intercrop	2.68±0.52	8.04±0.07	0.60±0.02	2.48±1.02	323.0±110.0	39.6±9.52
	Eugenia jambolana / Oat intercrop	2.78±0.60	8.02±0.04	0.75±0.03	8.62±2.20	330.2±77.7	51.2±1.02
Dry season	Oat Monocrop	1.60±0.10	8.10±0.03	0.71±0.06	12.48±2.56	222.4±4.60	50.0±1.41
	Mangifera indica / Oat intercrop	2.32±0.22	7.82±0.06	0.72±0.04	18.28±0.48	266.4±10.0	49.6±0.75
	Ficus benghalensis/ Oat intercrop	1.18±0.04	8.12±0.04	0.77±0.05	11.12±2.27	125.0±5.31	50.0±1.90
	Eugenia jambolana / Oat intercrop	10.0±0.26	7.66±0.02	0.84±0.02	9.26±0.67	730.0±11.9	50.4±1.17

Legend: EC= Electrical Conductivity; pH= Negative Log of Hydrogen Ion; OC= Organic Carbon Content; P= Available Phosphorus Content; K= Available Potassium Content; SP= Saturation Percentage. (Mean values ± Standard error)

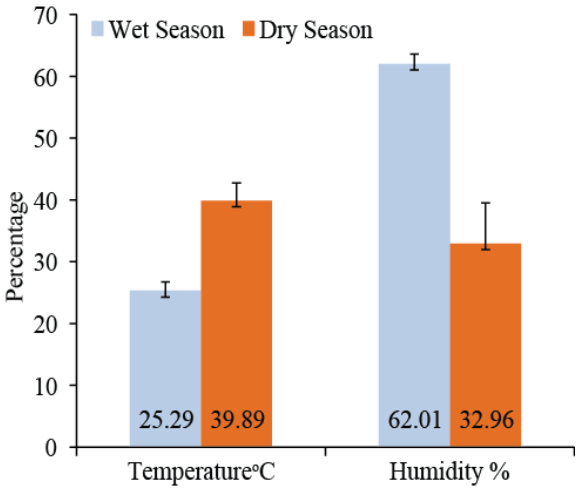

Fig. 1. Average temperature and humidity in wet and dry seasons at experimental site.

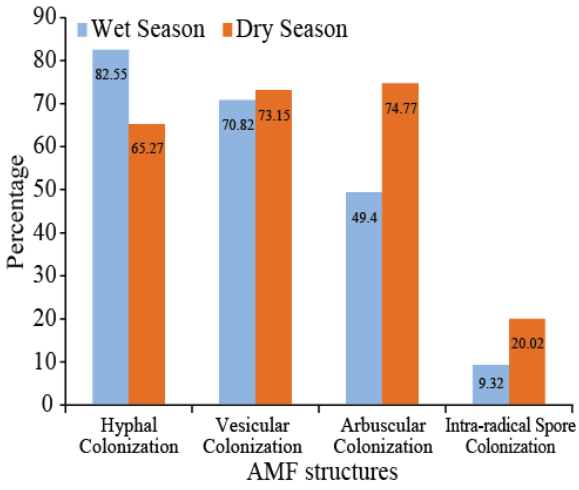

Fig. 2. Percentage of arbuscular-mycorrhizal fungal structures in wet and dry seasons.

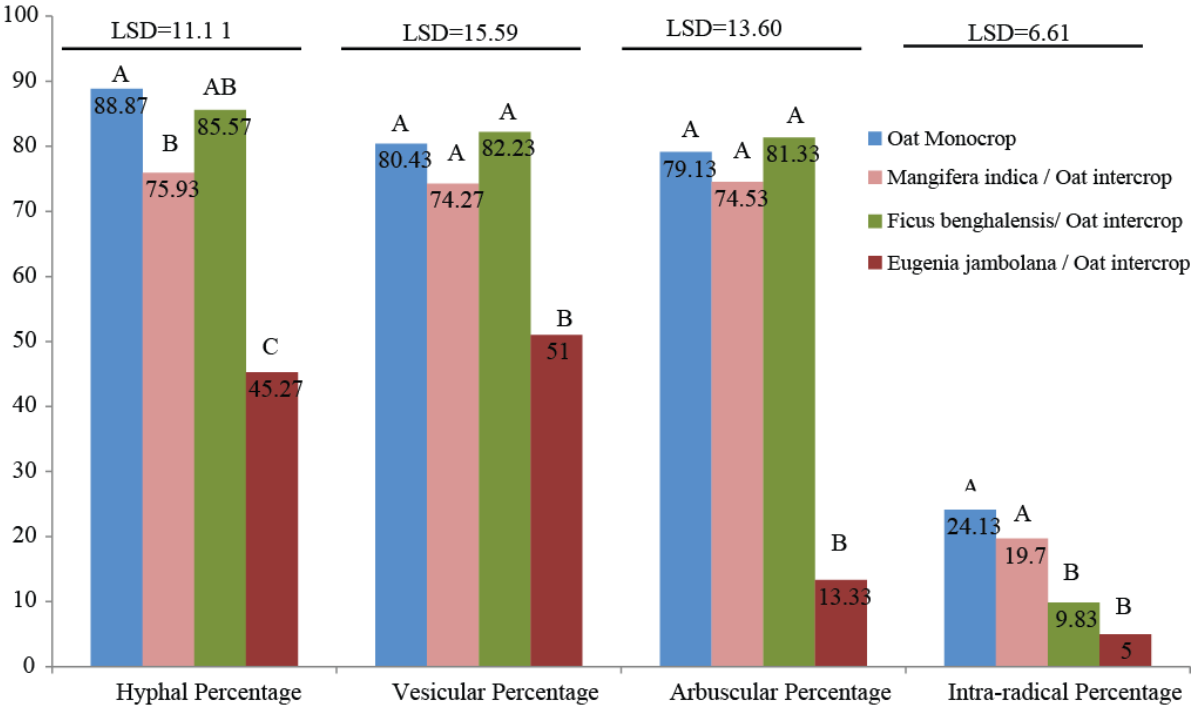

Fig. 3. Colonization status of AMF in four different cropping systems and their least significant difference.

Table 2 shows analysis of AMF variance in response to season and host cropping systems. The F-values shows that seasonal patterns have a strong significant effect on the hyphal percentage, arbuscular percentage and intra-radical spore percentage but have not showed any effect on vesicular percentage. Variation in host agroforestry system (host species aggregation pattern) also significantly alters AM fungal colonization. All AMF structures vary considerably in their occurrence in response to varying host cropping pattern. No combined effect of seasonality and cropping regimes on AMF colonization percentage has been assessed.

Pearson's correlation among different variables of arbuscular-mycorrhizal fungi and soil physico-chemistry showed interesting results in Table 3. Soils having high electrical conductivity were negatively correlated with AMF as well as with an important character of AMF colonizing soils, i.e. available phosphorus content. Secondly, pH showed a negative correlation with all physical and chemical properties of soil studied but no correlation with other AMF structures. The correlation analysis among AMF and organic carbon content was as expected. Organic carbon content enhanced the soil saturation percentage and decrease the occurrence of all

AM fungal structures. Similarly, saturation percentage decreases AMF percentage. Available potassium content showed negative correlation with soil pH, arbuscules and intra-radical spore percentage and significant negative correlation with hyphal and vesicular percentage. AMF structures also showed correlation with each other. Hyphal colonization showed a significant positive correlation with vesicular colonization status but no significant correlation with other AMF parameters. Arbuscular percentage in roots possess a strong significant correlation with vesicular and intra-radical spore percentage and vice versa.

The incidence of occurrence of AMF populations varied considerably among different cropping systems in both seasons (Table 4). In both wet and dry seasons, *Ficus benghalensis*/ Oat intercrop site possessed highest relative density followed by Oat monocrop, *Mangifera indica* / Oat intercrop and *Eugenia jambolana* / Oat intercrop site respectively. On the other hand the diversity of AMF was also highly variable among different cropping systems. Shannon-Weiner index showed highest AMF diversity in Oat monocrop in wet season and in *Ficus benghalensis*/ Oat intercrop in dry season. Overall, diversity index was high in dry season as compared to wet season. Taking cropping variation into account, in wet season Oat monocrop and *Ficus benghalensis*/ Oat intercrop possessed same diversity index followed by *Eugenia jambolana* / Oat intercrop and *Mangifera indica* / Oat intercrop site. However, in dry season *Ficus benghalensis*/ Oat intercrop possessed highest AMF diversity followed by Oat monocrop, *Eugenia jambolana* / Oat intercrop and *Mangifera indica* / Oat intercrop discerningly.

Table 2. F-values from repetitively measured ANOVA for Arbuscular-Mycorrhizal Colonization in root samples collected from four cropping systems in wet and dry season.

	Hyphal colonization	Vesicular colonization	Arbuscular colonization	Intra-radical spore colonization
Season	19.0630**	0.1763	27.3974**	20.6501**
Agroforestry systems	25.1889**	6.7151**	45.3145**	13.9337**
Season x Agroforestry system	0.3530	0.2689	0.5698	2.0228

** Correlation is significant at 0.01 level

Table 3. Pearson's correlation coefficients among physio-chemical soil parameters and Arbuscular-Mycorrhizal fungi.

	Electrical conductivity	pH	Organic carbon content	Available phosphorus	Avaliable potassium	Saturation percentage	Hyphal colonization	Vesicular colonization	Arbuscular colonization
pH	0.055								
Organic carbon content	0.565	**-0.163**							
Available phosphorus	**-0.194**	**-0.269**	0.289						
Avaliable potassium	0.635	**-0.657**	0.393	0.000					
Saturation percentage	0.142	**-0.069**	**0.831***	0.517	0.024				
Hyphal colonization	**-0.306**	**0.754***	**-0.631**	**-0.354**	**-0.798***	**-0.491**			
Vesicular colonization	**-0.512**	0.477	**-0.622**	0.097	**-0.739***	**-0.428**	**0.828***		
Arbuscular colonization	**-0.488**	0.221	**-0.412**	0.308	**-0.594**	**-0.231**	0.602	**0.926****	
Intra-radical spore colonization	**-0.215**	0.090	**-0.126**	0.634	**-0.305**	0.160	0.251	0.595	**0.744***

*= Correlation is significant at the 0.05 level (2-tailed)
**= Correlation is significant at the 0.01 level (2-tailed)

Table 4. Relative spore densities and Shannon-Weiner diversity indices of arbuscular-mycorrhizal fungi in four different cropping systems in wet and dry seasons.

Cropping systems	Wet season		dry season	
	Relative density	Shannon-weiner index	Relative density	Shannon-weiner index
Oat Monocrop	25.71	1.21	26.71	1.30
Mangifera indica / Oat intercrop	20.52	1.03	17.07	1.08
Ficus benghalensis/ Oat intercrop	39.89	1.20	39.54	1.36
Eugenia jambolana / Oat intercrop	13.88	1.14	16.68	1.16

Discussion

The seasonality, soil physico-chemistry and different host cropping systems are critical factors which structure the AMF communities in soil and have a significant effect on the colonization status of Arbuscular-Mycorrhizal fungi in roots of host plants. The colonization status of AMF structures was significantly affected by seasonal variation but overall it was high in both wet and dry seasons. Sigu¨enza *et al.* (1996), Lugo *et al.* (2003) and Bohrer *et al.* (2004) reported AMF colonization to be highest in dry hot period of July and September. It's a general concept that arbuscular-mycorrhizal status is highest in dry hot period of the year (June and July) because it is growing stage for most agricultural plants. Whereas, Mohammad *et al.* (1998) stated it to be highest in winter in months of December and January. While inconsistent results were described by Brundrett & Abbott (1994) who publicized that seasonal fluctuations are not substantial for AM fungal colonization. Kennedy *et al.* (2002) also reported that AMF colonization could be correlated with growth stages of plants or stress

conditions like drought and rain, rather than harmonization with seasonal fluxes. Our results were little different that total AMF infectivity was almost same, however, fluctuations occurred in individual AMF propagule ratios. The percentage of hyphae, arbuscules and intra-radical spores varied significantly among wet and dry seasons but that of vesicles remained almost same. Arbuscular and intra-radical spore percentage was high in dry season as compared to wet season. It might be of the fact that the exchange sites (arbuscules) enhanced in stress condition and at extreme growth stage. Secondly, AMF sporulation also increased in stress alleviation modes (Evelin *et al.*, 2009). In contrast, hyphal percentage decreased in dry season showing a negative correlation with plant growth. Anjum *et al.* (2006) supported our results by reporting a decrease in mycelial network with root/shoot growth.

AM fungal status was significantly varied with deviation in cropping regimes. Similar outcomes were described by Ruotsalainen *et al.* (2002) that arbuscular mycorrhizal symbiosis is host-specific. The cropping pattern can employ a significant impact on the AMF populations (Alguacil *et al*., 2014). The treatment combining agricultural practices with forestry appears to be most appropriate agricultural organization strategy because it improves AM fungal diversity and colonization ability under tropical conditions like Pakistan climatology. This concept is supported by the findings of Carvalho *et al.* (2010) who described that agroforestry cropping systems have the potential to exploit the benefits connected with arbuscular mycorrhizal fungi, which in turn can alleviate negative relations between trees and crops.

AMF show spatial and seasonal dynamics. It's a general observation that AMF colonization and ecological factors are significantly correlated with each other. This estimation is strongly supported by Muthukumar & Udaiyan (2002) and Staddon *et al.* (2003). Our results supported the concept that soil heterogeneity had significant influences on AMF colonization patterns. Lingfei *et al.* (2005) also established same opinion with the exemption of vesicular colonization percentage which was not correlated with soil chemistry in his studies. Previous studies have revealed that salinity can decrease AM fungal colonization by preventing spore germination (Carvalho *et al.,* 2001). This aspect supported our findings, i.e. the AM fungal colonization status was negatively correlated with electrical conductivity of soil (Abeer *et al*., 2015). But some authors illustrate the contradiction of this assessment that the AMF sporulation is roused under salt-stress conditions (Hirrel, 1981). A significantly positive correlation was evaluated between arbuscular mycorrhizal mycelial colonization status and pH supported by Mathur & Vyas (1997). Gupta *et al*. (2002) braced the view that available phosphorus and AMF show a contradictory relationship, but some authors pointed out that rise in phosphorus could promote AMF and its decline might inhibit AMF (Xu *et al.,* 2008; Jia *et al.,* 2004). However, our research have showed no correlation between available phosphorus content and AMF status, supported with the findings of Ruotsalainen *et al.* (2002) and Bohrer *et al.* (2004) who sketched that occasionally AM fungi do not correlate with soil P status, especially when phosphorus content is not sufficient to suppress its colonization in host plants. A negative correlation among wet season and AMF colonization are in accordance with the findings of Muthukumar & Udaiyan (2002) but not consistent with the results of Braunberger *et al.* (1994) who reported a positive correlation among precipitation and AMF percentage. Soil saturation percentage has been reported by many authors to be positively correlated with AMF colonization including He *et al.* (2002). It may be a strong argument opposing our results, as soil saturation percentage is a vital factor in agricultural ecosystems. Organic carbon content and saturation percentage were positively correlated with each other that accords with the findings of Mohammad *et al.* (2003) but both showed a negative correlation with AMF colonization. Dhillon & Zak (1993) also acknowledged that plants growing in soils having high organic nutrient level, should expect a negative response as low AMF colonization and vice versa.

Conclusion

The study determined that change in seasonality, host plants composition of cropping system and soil chemical heterogeneity significantly alter AMF colonization status and diversity. AMF root colonization and rhizospheric spore densities were high in dry season as compared to wet. With respect to cropping regimes, mono-cropping sites showed highest root colonization than agroforestry cropping systems, whereas, highest AMF spore densities have been assessed in agroforestry sites rather than mono-cropping sites. Soil chemical heterogeneity also had significant influences on AMF colonization patterns in both seasons. This valuable relationship between AMF and different cropping patterns is depicted as a unique form of symbiosis and deserves more attention for in-depths study.

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