Final Evaluation Report

Your Details	
Full Name	TCHAN ISSIFOU Kassim
Project Title	Impact of human actions on the conservation and preservation of the functional diversity of Arbuscular Mycorrhizal Fungi (AMF) in Benin
Application ID	40556-1
Date of this Report	02/03/2025

1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Ethnoecological surveys to identify potential AMF threats			X	Using a Kobocollect survey form and audio recordings, interviews were conducted with 465 resource persons (farmers and foresters) in and around the nearest villages (Toui, Kilibo, Kokoro or Challa-Ogoi), with 155 interviewers per village. The questionnaire was designed to take account of socio-soil variables and certain questions, such as gender, age, marital status, socio-ethnic groups, level of education, length of land use, land management methods and cropping history. The questionnaire also took into account other aspects such as the type of fertilizers used, the amount applied per hectare, the pesticides used and their quantity, and stakeholders' perception of the impact of land management methods on the soil mycobiome. Analysis of the survey data yielded some interesting results. It should be noted that the majority ethnic groups are Nago (32.48%), Tchabè (11.46%), Fon (9.87%), Ditamari (7.64%), Lokpa (4.78%), Peulh (1.59%) and the other minority ethnic groups (32.17%), which accounted for less than 1%. All the people surveyed farm. Apart from farming, some people also engage in commerce (10.83%), handicrafts (8.92%), charcoal-making (1.27%), hunting (0.64%) and other small-scale activities (12.1%). The majority of respondents were aged between 25

				and 60 (94.27%), followed by those over 60 (3.82%) and under 25 (1.91%). A total of 09 endogenous farming practices were identified during our surveys: perpendicular slope ploughing (98.41%), crop rotation (95.86%), agroforestry (95.86%), animal stabling (95.54%), crop association (82.08%), crop residue management (81.53%), slash-and-burn agriculture (27.07%), stone cordon (1.59%), Direct seedingt (0.96%). In the field, all the people interviewed did not apply a single cultivation practice, but rather a combination of the cultivation practices mentioned above. For this reason, thanks to the monitoring of growers in their respective fields, we recorded 12 measures or combinations of endogenous cultivation practices. Based on field monitoring, twelve combinations were recorded: • C1 or PLA: perpendicular slope ploughing + Livestock pasture + crop association (4.79%) • C2 or RCM: Crop rotation + Crop residue management (83.39%) • C3 or CRM: Crop residue management only (3.83%) • C4: perpendicular slope ploughing + Crop residue management + Livestock pasture (3.51%) • C5: Crop residue management + Livestock pasture + Crop rotation (0.64%) • C6: perpendicular slope ploughing + Agroforestry + Crop residue management + Livestock pasture (1.28%) • C7: stone cordon + crop association + slash-and-burn

				agriculture (0.32%) • C8: crop association + slash-and-burn agriculture + Livestock pasture (0.32%) • C9: Livestock pasture (0.32%) • C10: perpendicular slope ploughing + Livestock pasture + stone cordon (0.32%) • C11: Crop rotation or succession + crop association (0.32%) • C12: crop association + Crop residue management + stone cordon + Livestock pasture (0.96%) The main crops in the area are maize (99.68% of respondents), cassava (75.8%), and yam (71.02%). In maize production, the dominant crop, 78.34% of producers use mineral fertilizers (urea and NPK), 4.46% use organic fertilizers (cow manure), and 17.2% apply no fertilizers. Regarding weed control, 58.92% of farmers use herbicides, while 41.08% prefer manual weeding. As for local perceptions, 74.84% of respondents believe that their farming practices negatively affect the conservation and proliferation of soil mycobiomes, whereas only 0.96% disagreed. The remaining 23.89% held neutral opinions on the subject. Finally, direct observations along 90 transects (30 per village or management unit), conducted under the supervision of the local forest administration, confirmed various human threats to the natural habitats of arbuscular mycorrhizal fungi (AMFs). Additional observed disturbances included cut stems, illegal logging, conversion to cropland, firewood collection, charcoal production, and

				overgrazing of AMF-host trees (through pollarding, debarking, pruning, etc.) in each of the three management units.
Diversity and distribution of AMFs according to the different land-uses			X	For soil sampling in agricultural fields, the three combinations of cultivation practices most commonly used by the local population were considered (C1, C2 and C3). In each management unit, soil samples were taken from 15 sites: 03 sites for C1 or PLA, 03 sites for C2 or RCM, 03 sites for C3 or CRM, 03 sites for natural forest and 03 others in fallow land near fields at least 5 years old. A total of 45 sites were considered for soil sampling in the three management units (Challa-Ogoi; Toui and Kilibo). For the natural forest and fallow sites, a 2500 m² plot was installed at each site, while for the agricultural field sites (PLA, RCM and CRM), 5 plots of 100 m² were installed, 4 at the ends and 1 in the middle. In each plot, soil samples were taken at each corner and in the middle of the plot. In fact, 25 soil cores (05 to 15 cm soil depth representing the zone of intense AMF activity) were sampled at each site using an auger. The different soil cores were mixed at each site to form a composite sample. A total of 45 composites samples were formed for the three development units, with 15 composite samples per zone or development unit. AMF spores were isolated from 100 g of each composite soil sample (per site) using the technique described by Chabi bogo et al. (2020) using 700 µm, 300µm, 200µm and 40 µm sieves. Spores were distinguished into morphotypes using color, shape, size, attachments and surface, presence or absence of

				hypha and their identification was carried out by comparison with the descriptions of Jefwa et al. (2012) and the identification sites (http://www.i-beg.eu/ and https://invam.wvu.edu/). Identified spores are then preserved for future research by adding glutaraldehyde. A total of 31934 spores were isolated from 45 composites soil samples, divided into 24 species and 8 genera (*Acaulospora*, *Ambispora*, *Funneliformis*, *Gigaspora*, *Glomus*, *Rhizophagus*, *Scutellospora*, *Septoglomus*) (See AMFs genera photos). The results of the ANOVA test show that mean spore abundance varied significantly from one cropping system to another (p=0.000025914), in contrast to the different localities or management units sampled (p=0.78). The highest mean spore abundance values were recorded under the fallow system (834 spores), in contrast to the C3 or CRM cropping practice (588 spores), which obtained the lowest mean density (figures 1, 2 and 3). The ANOVA test also showed a significant difference in AMF diversity between cropping systems (p-value=0.038199). However, this diversity was not significantly different between the different localities (p-value=0.600943). As for the average abundance of AMF species, it varied significantly from one species to another on 100 g of soil sampled (p-value=0.0029). Indeed, the distribution of AMF species over 100 g of soil (Figure 4) reveals that species such as *Glomus* sp1. (173 spores), *Acaulospora* sp1. (116 spores) and *Gigaspora* sp1. (76 spores) are more dominant, in contrast to

				species such as *Septoglomus* sp1. (1 spore), *Rhizophagus aff. irrégularis* (1 spore) and *Septoglomus aff. deserticola* (2 spores). The radar diagram shows that Forest and Fallow favor both high abundance and proliferation of AMF species. Among the different cropping systems, (Forest), (Fallow), PLA and RCM stand out with a high dominance of *Glomus* sp1., in contrast to CRM, which stands out with a high density of *Acaulospora* sp1.
Identification of rare or threatened AMF strains			X	The identification of rare species was followed by estimates of the Jackknife 1 and Jackknife 2 indices, which gave values of 26.93 and 29.86 respectively (figure 5). These estimates indicate a higher number of potential species than those directly observed, suggesting the presence of rare species (Figure 5). By cross-referencing these results with the presence/absence analyses, the species considered to be rare, i.e. present in only one or two samples, were *Septoglomus* sp1. (present in 1 sample), *Rhizophagus aff. intraradices* (present in 1 sample), *Acaulospora aff. mellea* (present in 1 sample), *Glomus aff. fecundisporum* (present in 2 samples), and *Rhizophagus aff. irrégularis* (present in 2 samples). The various strains of these rare species are kept in our inoculum bank at the University of Parakou.
Raising farmers' awareness of best agricultural practices for the conservation of natural AMF habitats.			X	Using leaflets, posters, bags, animated debates in local languages (Nago, Tchabè and Fon), and publicity posters, we raised awareness among around 450 people over the course of nine sessions (3 sessions per village) about the importance of safeguarding the

				Toui-Kilibo Classified Forest and showed the population how the pressure exerted on this forest affects the diversity of AMFs (see photos below). Our discussions also focused on the ecological role of AMF, the importance of preserving their habitats in order to maintain the ecosystem services they provide, and the good agricultural and forestry practices that encourage their proliferation. The various stakeholders are farmers, loggers, village forest management associations, youth and women's groups and local NGOs involved in forest management. These various awareness-raising activities have made it possible to strengthen socio-ecological resilience by consolidating sustainable interactions between local communities and forest ecosystems, in particular through the preservation of arbuscular mycorrhizal fungi (AMF), the pillars of soil fertility and forest health. By raising awareness among forestry officials and local populations of sustainable soil management practices, the project is improving the capacity of socio-ecological systems to adapt to disturbances such as climate change, deforestation and land degradation.

2. Describe the three most important outcomes of your project.

a). First study on the diversity of AMFs in the target forest

The Toui-Kilibo classified forest is home to a mycobiome of 29 species, 24 of which have been identified in 8 different genera. It should be noted that the identification of AMF strains is fairly complex, and 8 of the 24 species were determined down to genus level. The results of the ANOVA test indicate that AMF diversity (abundance and species richness) is influenced by cropping systems. The radar diagram shows that Fallow and Forest favour both high abundance and proliferation of AMF species. Of the three endogenous cropping practices practised by the local communities, only the PLA practice preserves AMF diversity (abundance and species richness) better than the other two (RCM and CRM).

b). Identification of rare or threatened species
Taking into account the estimators and the presence/absence data for the various sites sampled, a total of 5 species were identified as rare out of the 24 species. These were species such as *Septoglomus* sp1, *Rhizophagus aff. intraradices*, *Acaulospora aff. mellea*, *Glomus aff. fecundisporum*, and *Rhizophagus aff. irrégularis*.

c). Provision of an inoculum bank
A total of 31934 spores were isolated from the 45 composite soil samples. The various spores isolated were processed and stored in an inoculum bank at the Mycothèque of the University of Parakou for future scientific work and comparison.

3. Explain any unforeseen difficulties that arose during the project and how these were tackled.
During the implementation of this project, two major difficulties were overcome. Firstly, the ethnoecological surveys were scheduled to be carried out during the period June to August 2023. Unfortunately, funds were not readily available due to a small problem in the transaction process which extended this activity from August to October 2023 and altered the rest of the project timetable. Secondly, the laboratory refurbishment work undertaken in early 2024. These various works, which took more than 6 months, not only led to the closure of certain spore handling and isolation areas, but also deprived the laboratory of the water essential for spore sieving, isolation and processing activities.

4. Describe the involvement of local communities and how they have benefitted from the project.
The local population living near the Toui-Kilibo classified forest has shown a willingness to take part in all the activities carried out as part of this project. This can be explained by the active involvement of local communities during ethnoecological surveys, transects within the target forest, participatory awareness-raising sessions and information campaigns adapted to socio-cultural realities. The results of the analysis of the survey data provided by the local communities enabled us to find out their level of knowledge about AMFs and also to know that 75% of the respondents thought that their soil management methods had a negative effect on AMFs (but did not know how), unlike 24% of the farmers who had no perception or knowledge of arbuscular mycorrhizal fungi. The results of the surveys also enabled us to identify the poor farming practices adopted by local people. Thanks to this key information, more than 450 members of the local community were able to increase their knowledge of the ecological importance of AMFs and the impact of their actions on the conservation and sustainable management of this fungal resource. In addition, this information enabled us to emphasise the ecosystem services provided by CMAs and the importance of conserving them during our awareness-raising activities with local communities. During our awareness-raising sessions, the local community was told about the favourable habitats and good cultivation practices necessary for the proliferation of CMA. Finally, posters containing photos of CMAs were made available to local communities to relay the information to other growers who were unable to attend the sessions.

5. Are there any plans to continue this work?
The Toui-Kilibo Classified Forest is a unique and special ecosystem, as it represents the largest dense dry forest (43,350 ha) in Benin. Of the 29 possible species, only 24 provisional species have been identified, including 5 threatened or rare species. This means that, as a first step, we urgently need to carry out large-scale trap cultures (from the soil) and single-spore cultures (from each rare strain) of these different threatened strains in our future work, with the aim of producing inocula or biofertilisers for application in the field. This will not only contribute to the long-term conservation of these rare species in the field, but also help to restore them. Secondly, to carry out molecular analyses of pure strains of traps and monosporal cultures in order to obtain reliable identification of AMF species and to detect or reveal potential new species for Benin. This will enable a catalogue of AMF species for Benin to be compiled. The PLA practice emerged as the best endogenous cultivation practice that promotes the sustainable proliferation and conservation of AMFs. To this end, our future work must take into account the installation of demonstration sites or experimental fields of best cultivation practices at the level of each unit in order to restore and better conserve AMFs. In the field, it was observed that trees were being felled in the heart of the forest, indicating a need to reforest the forest with some typical trees such as *Pterocarpus erinaceus*, *Isoberlinia* sp, *Afzelia africana* and *Khaya senegalensis*. These trees will be inoculated with strains of rare AMFs that have been propagated with the aim of restoring AMF habitats for their sustainable management.

6. How do you plan to share the results of your work with others?
The internal rules of the Tropical Mycology and Plant-Soil Fungi Interaction (MyTIPS) research unit require that at the end of each project carried out, each project leader organises feedback sessions for the other internal and external auditors of the laboratory. A feedback session will therefore be organised in the Salle des Professeurs of the Faculty of Agronomy at the University of Parakou for undergraduate and postgraduate students. This session will provide all the Faculty's researchers and students with the same level of information. It will also promote arbuscular mycorrhizal fungi and encourage their conservation and sustainable management in Benin. Thanks to the illustrative data acquired during this project, we are producing brochures and posters illustrating the diversity of AMFs under the different management units. These tools will be used as teaching aids during our feedback sessions and discussions on the biological life of soils with secondary school students, producer groups in the project area, technicians from the Agence Territoriale pour le Développement Agricole (ATDA), and NGOs responsible for forestry management in the study area. The aim of these exchanges will be to draw their attention to the ecological importance of AMFs and the need to integrate this overlooked underground biodiversity into future conservation actions. At the international level, the results will be published in a peer-reviewed international journal, preferably with open access in order to share the results as widely as possible with a large community of scientists.

7. Looking ahead, what do you feel are the important next steps?
As already mentioned above, the Toui-Kilibo Classified Forest is a unique ecosystem with 5 threatened species due to strong population pressure. In addition, many of the natural habitats of the AMFs in the forest are being degraded, not only by poor

agricultural practices but also by the felling of threatened host trees such as *Afzelia africana*, *Khaya senegalensis*, *Vitellaria paradoxa* and *Pterocarpus erinaceus*. Urgent activities for the continuation of this project include (a) Trap culture and single-spore culture of the various endangered or rare strains, with the aim of producing large-scale inocula or biofertilisers for use in the field; (c) reforestation of degraded forest habitats with a few typical trees (*Pterocarpus erinaceus*, Isoberlinia sp., *Afzelia africana*, *Khaya senegalensis*) inoculated with AMF strains.

8. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

The Rufford Foundation logo was used throughout the project. Firstly, when the project was awarded, we published it on our laboratory's Facebook page and the foundation's logo was featured prominently. In addition, we published posters for the ethnoecological surveys, and the logo was also used. Lastly, the logo was used on t-shirts, bags, leaflets and posters used in campaigns to raise awareness of the ecological importance of AMFs.

The Rufford Foundation did not receive any publicity, but we made sure that the Foundation was well represented and highlighted in all the activities and documents produced.

9. Provide a full list of all the members of your team and their role in the project.

Tchan Issifou Kassim: PhD in mycobiomes of agricultural and forest soils from the University of Parakou. Facilitate the coordination of all project activities. Participate in all project activities.

Mr Joseph: He's a local guide who knows the Challa-Ogoi development unit very well. He is originally from Challa-Ogoi and speaks the local language. He has been involved in implementing all the project's activities in the field.

Mr Médard: He's a local guide who knows the Kilibo development unit very well. He is originally from Kilibo and speaks the local language. He has been involved in implementing all the project's activities in the field.

M. Clément: He's a local guide who knows the Toui development unit very well. He is originally from Toui and speaks the local language. He has been involved in implementing all the project's activities in the field.

Mme Chabi Bogo Taïbatou: PhD student at the University of Parakou, working on mycorrhizal fungi of cultivated plants. She was a key agent in the isolation, counting and identification of AMFs.

TAMMOU Omar: Holds a Master's degree from the University of Parakou and is a field agent. He was an important asset during soil sampling.

TORE Eric: Bachelor's degree from the University of Parakou. He was a good field agent for identifying and recording data on the current state of AMF natural habitats in the study area.

Bio Oumourath: Bachelor's degree in sociology from the Department of Sociology and Anthropology of the Faculty of Letters, Arts and Human Sciences. Her expertise was useful in guiding the awareness-raising activities of the various stakeholders and local players.

GOUTON Morissonne: PhD in planning and management of natural resources from the Faculty of Agronomy at the University of Parakou. He has played a key role in raising awareness among local communities and in data processing and analysis.

10. Any other comments?

We would like to thank the Rufford Foundation for its financial support for this project on sustainable management and biodiversity conservation in Ouèssè and Tchaourou.

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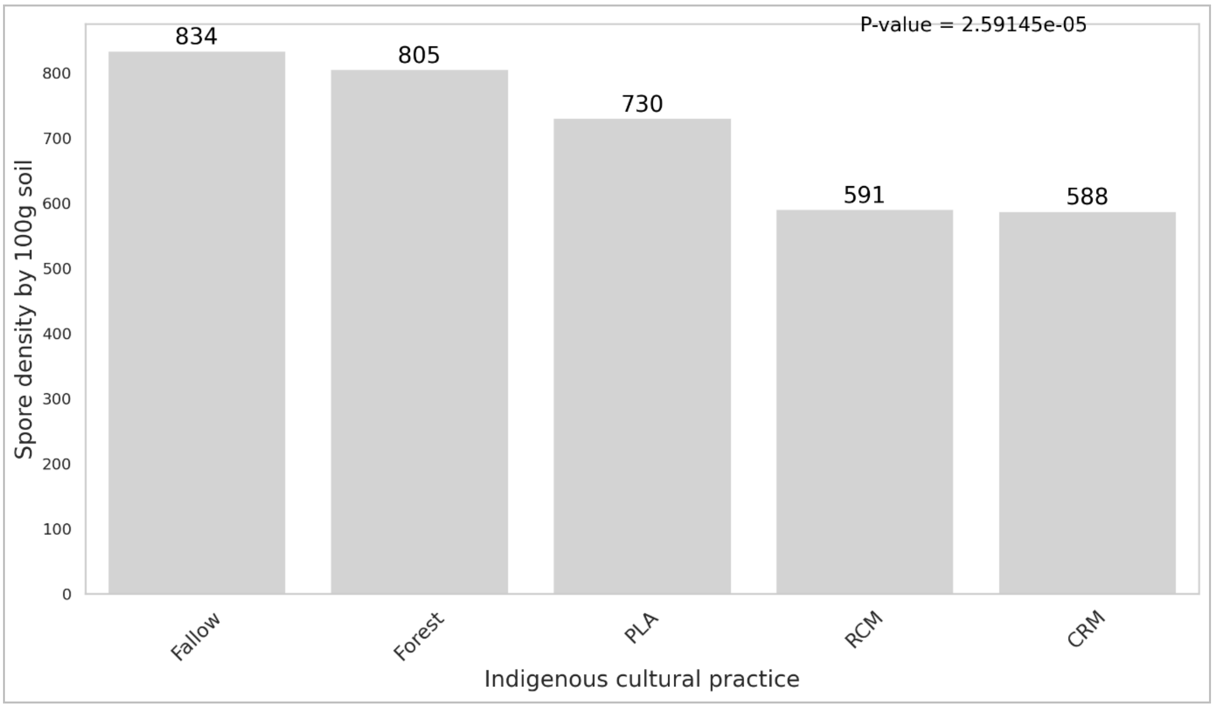


Figure 1: Average spore abundance according to forest soil management modes

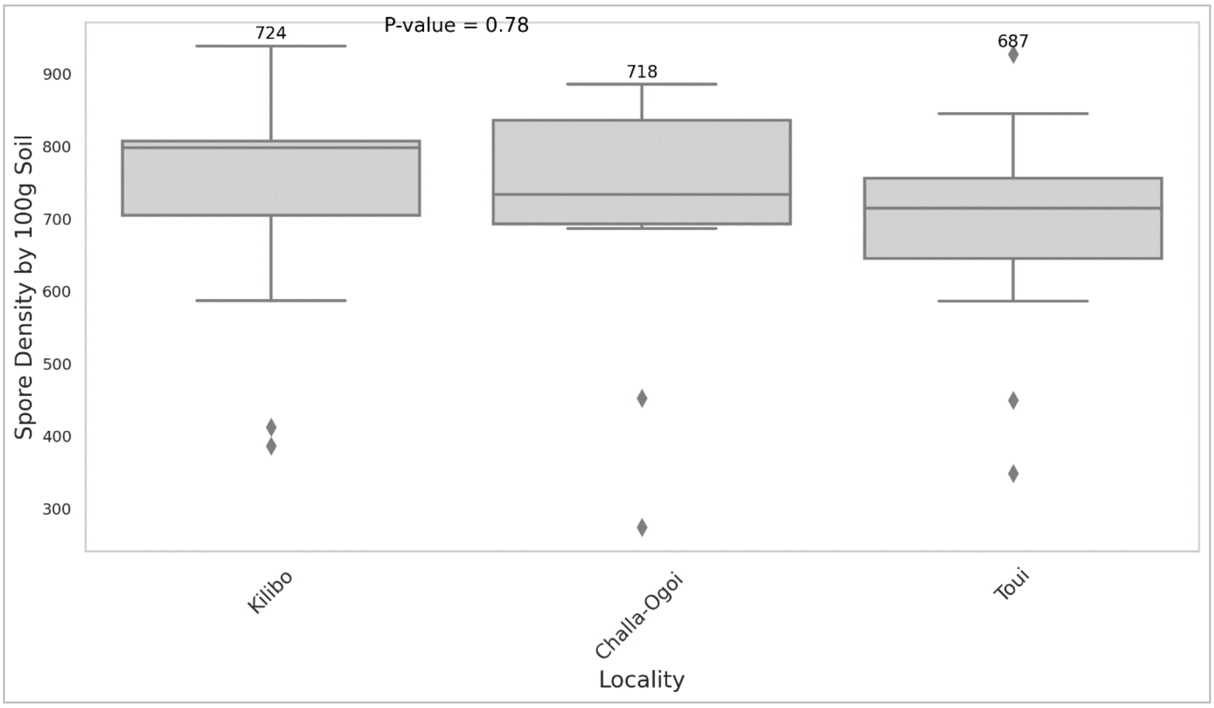


Figure 2: Average spore abundance by sampling locaties

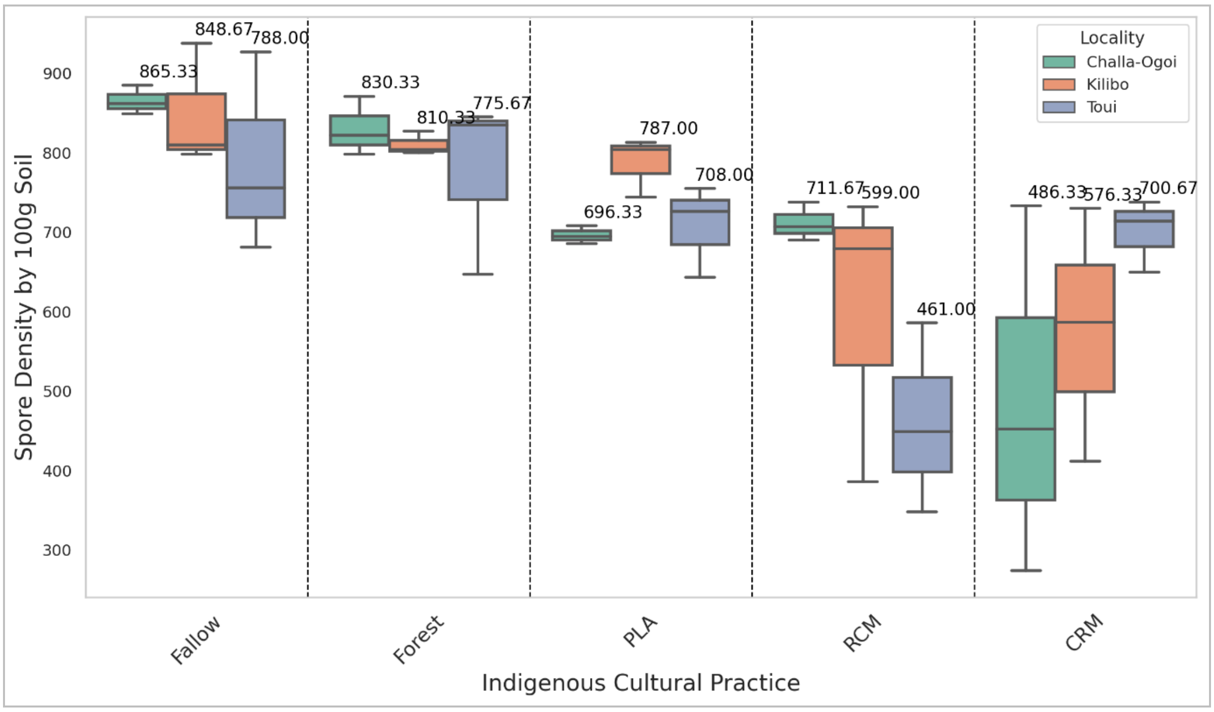


Figure 3: Average spore abundance per locality according to cropping system

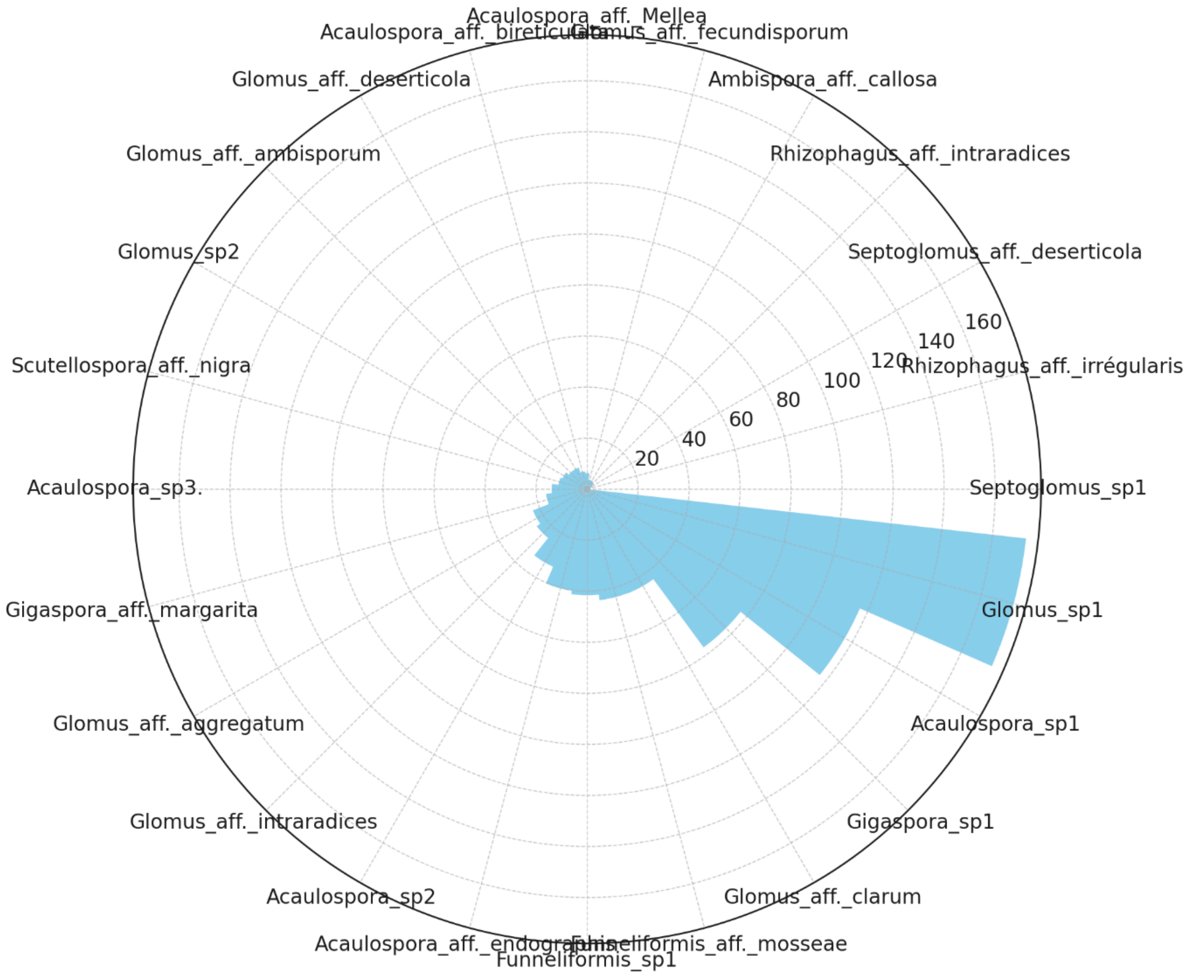


Figure 4: Dominance of AMF species per 100g of soil

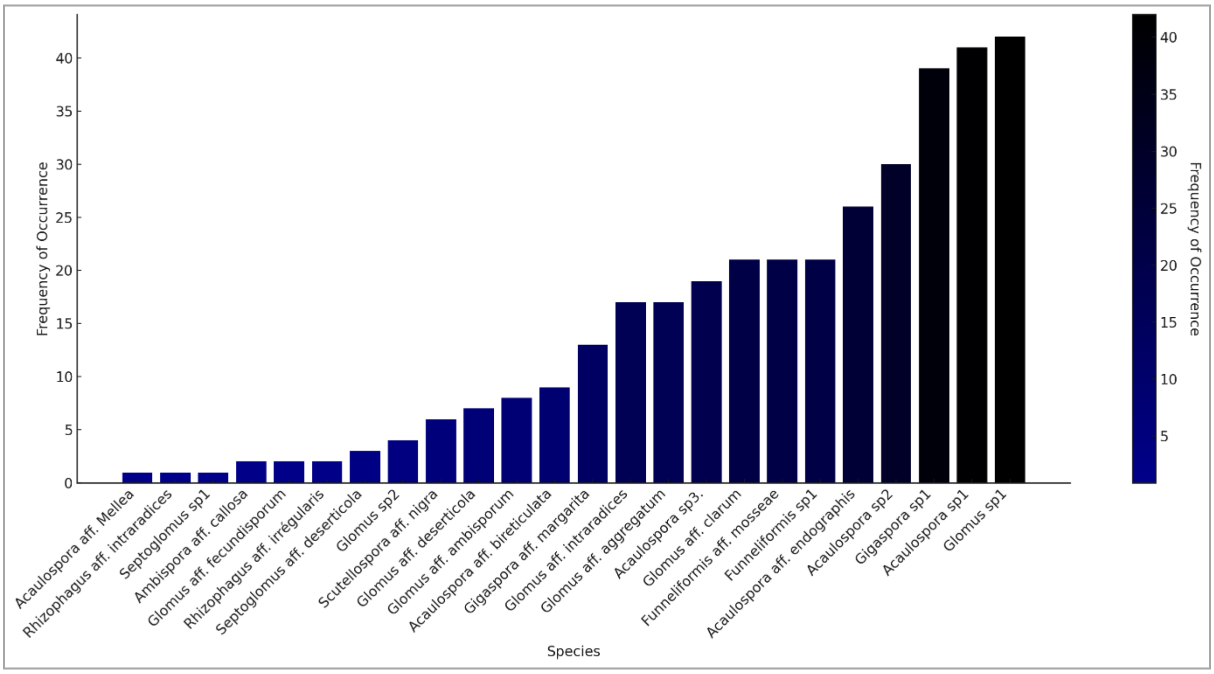
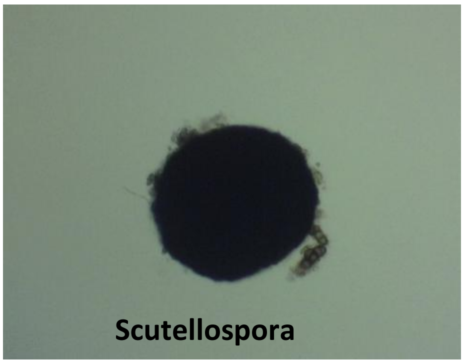


Figure 5: Classification of species according to rarity on the basis of their frequency of appearance in samples

Photos board of the different of AMFs genera


Acaulospora


Gigaspora

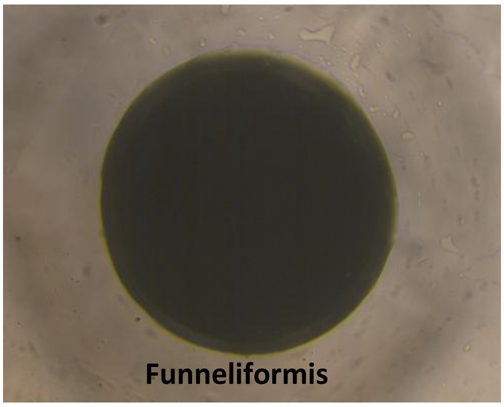

Scutellospora


Rhizophagus


Septoglomus


Glomus


Ambispora


Funneliformis


Diversity and importance of AMFs for crops
Generality of AMF
Diversity of AMFs
Diversity of arbuscular and vesicular mycorrhizae
Importance of AMFs for cultivated plants
Acknowledgments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The Rufford Foundation


S6P3
S6P4