DETAILED FINAL REPORT

Impact of human actions on the conservation and preservation of the functional diversity of Arbuscular Mycorrhizal Fungi (AMF)

in Benin

Introduction

Despite being known to play a disproportionate role in global ecosystem services, including regulating climate and the water cycle and maintaining biodiversity, tropical forests are under constant threat from deforestation and degradation driven by the expansion of agriculture, extractive industry and infrastructure (Bebbington et al. 2018). Human pressure on tropical forests will increase, underlining the need to conserve high quality habitats, particularly in global biodiversity hotspots. In Benin, more specifically in the Toui-Kilibo (TK) classified forest, a diachronic study of land use showed a regression of woodlands forests (32%), dense forests (10%) and gallery forests (30%) to the detriment of crop and fallow land (766%) and built-up areas (85%) (Igué et al. 2010). This drastic degradation of the forests is due not only to the illegal felling of trees of economic importance, but above all to inappropriate cultivation practices, such as the use of chemical inputs (Dewitte et al., 2012). Fortin et al (2008) and Willis et al (2013) have shown the harmful effect of poor agricultural practices (pesticides, chemical fertilisers) not only on soil conservation and its ecosystem services (pollution of agricultural products, food insecurity) but also on the colonisation and sporulation of soil micro-organisms and their disappearance. Among these soil micro-organisms that are vulnerable to human action are arbuscular mycorrhizal fungi (AMF), for which even moderate chemical input reduces spore density by 50% over five years (Gosling et al., 2006; Tedersoo et al., 2021). Despite their crucial importance in ecosystem functioning by improving growth and mineral nutrition parameters and protecting plants from pathogens through mycorrhizal symbiosis, all conservation projects in Benin have concentrated on above-ground or aerial fungi (Rufford 30193-1; Rufford 28216-1; Rufford 36954-B), forgetting about underground fungi. The effects of human activity on above-ground biodiversity (macromycetes) are physically observable, and so all conservation efforts are directed at this biodiversity. However, no studies have been carried out in Benin to assess the extent to which subterranean biodiversity, particularly 'physically invisible' CMAs, is also being damaged by human activities, in order to justify government conservation policies and programmes. The aim of this project is to document the impact of different forest soil management methods (field, fallow and natural forest) on the functional diversity of AMFs. Thanks to the quantitative and qualitative data collected as part of the project, preferential habitats for AMFs will be identified and used to draw up management guidelines for conservation practitioners.

Project objective

The ultimate objective of this project is to document the impact of different forest soil management methods (agricultural fields, fallow lands and natural forests) on the functional diversity of AMF. More specifically, this will involve:

(i) Conducting an ethnoecological survey among farmers to record all endogenous agricultural practices that are threatening the survival of AMF,

(ii) Investigating the diversity, distribution and abundance of AMF according to the different land-uses and the major agricultural methods recorded,

(iii) Identifying the rare and threatened AMF strains to conserve them in an ex-situ bank of spores,

(iv) Raising awareness to farmers on the best agricultural practices for conserving the natural habitats of AMF.

Activities

1. Ethnoecological surveys to identify potential AMF threats

Using a Kobocollect survey form and audio recording, 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 (August to October). 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:

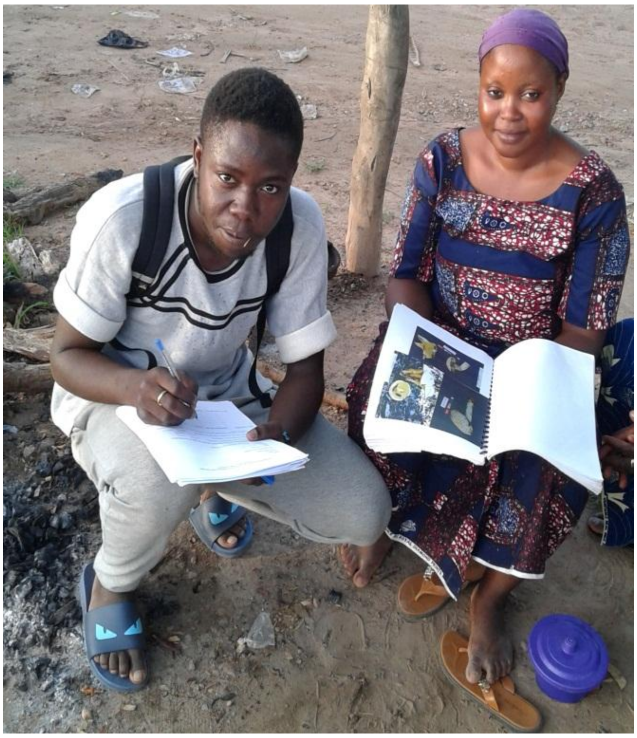
perpendicular slope ploughing (98.41%), crop rotation (95.86%), agroforestry (95.86%), animal stabling (95.54%), crop association (82.08%), crop residue management (81.53%), slash-and-burn agriculture (27.07%), stone cordon (1.59%), Direct seedingt (0.96%). In the field, all the people interviewed did 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.



Photos : Ethnoecological surveys

2. **Soil sampling**

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.

Photos: Soil sampling

3. Isolation and preservation of AMFs spores

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.

Sieves

Spores' isolation

Treated soils **Conservation of isolated spores**

4. Diversity and distribution of AMFs according to the different land-uses

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*). 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.*

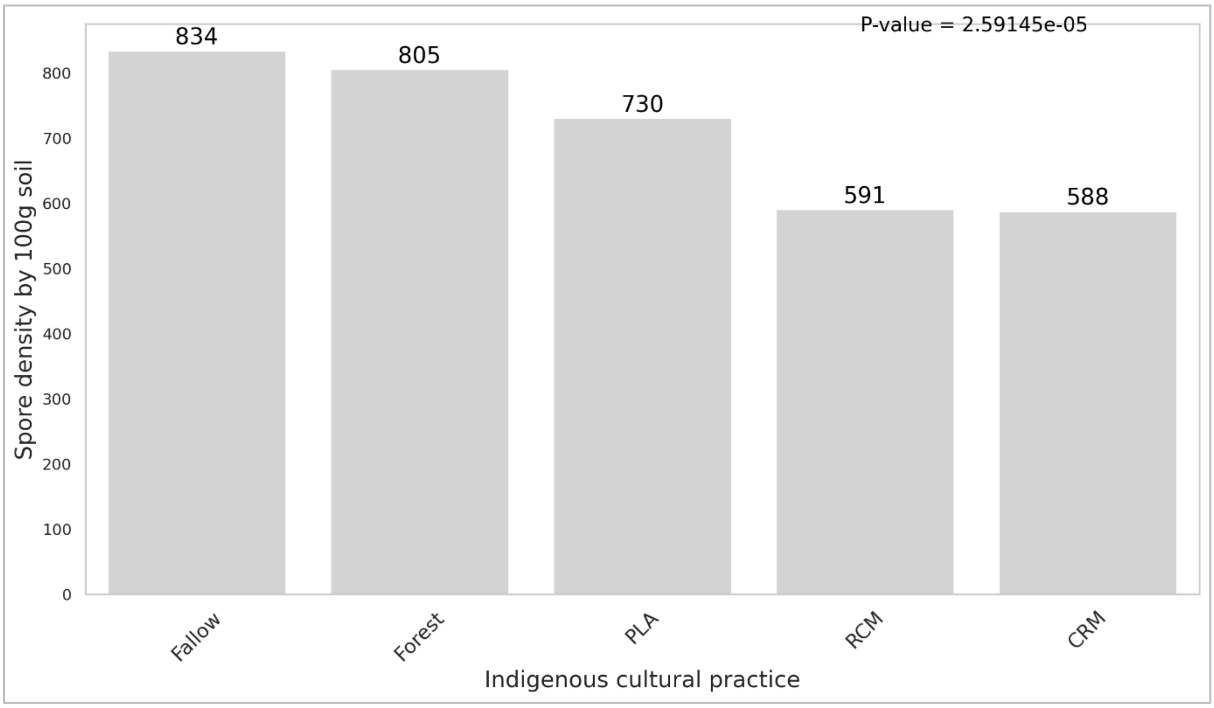


Figure 1: Average spore abundance according to forest soil management modes

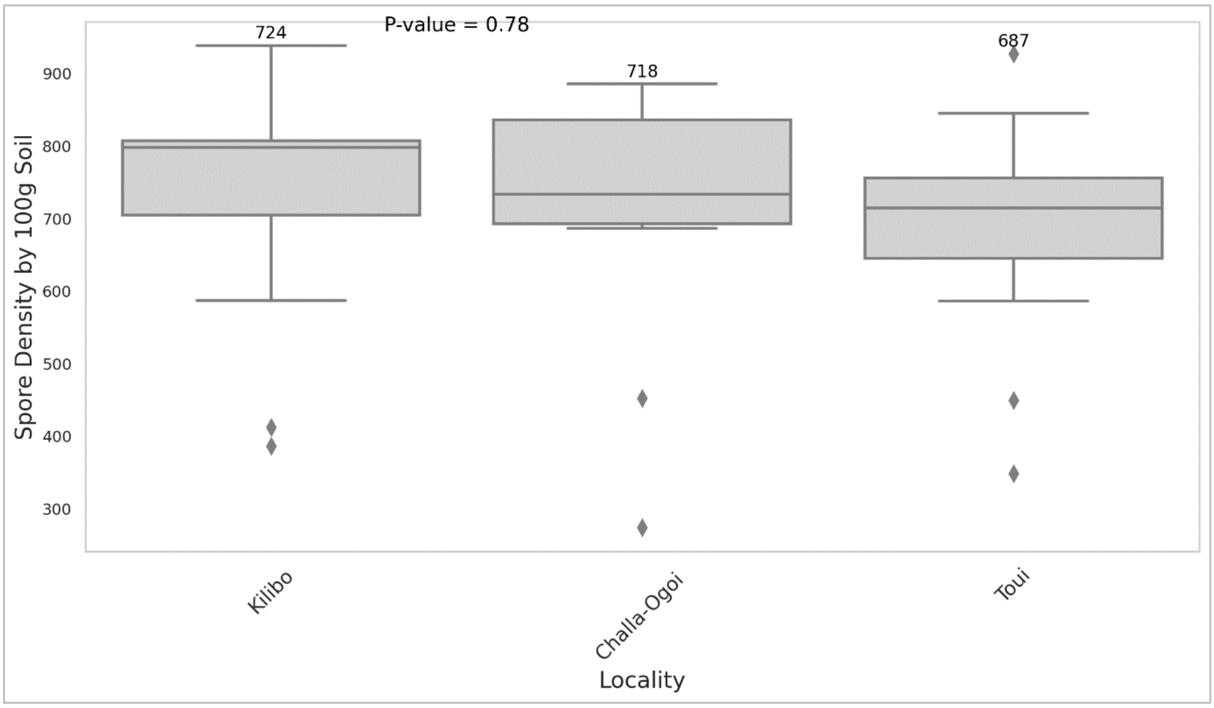


Figure 2: Average spore abundance by sampling locaties

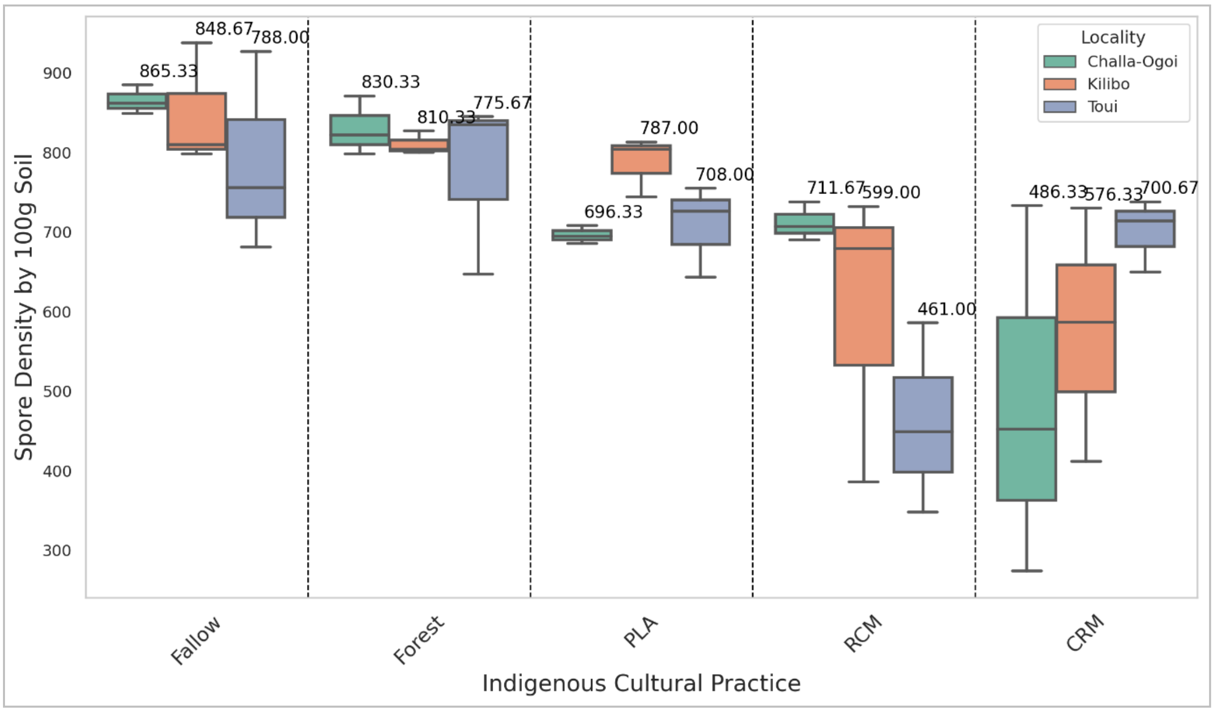


Figure 3: Average spore abundance per locality according to cropping system

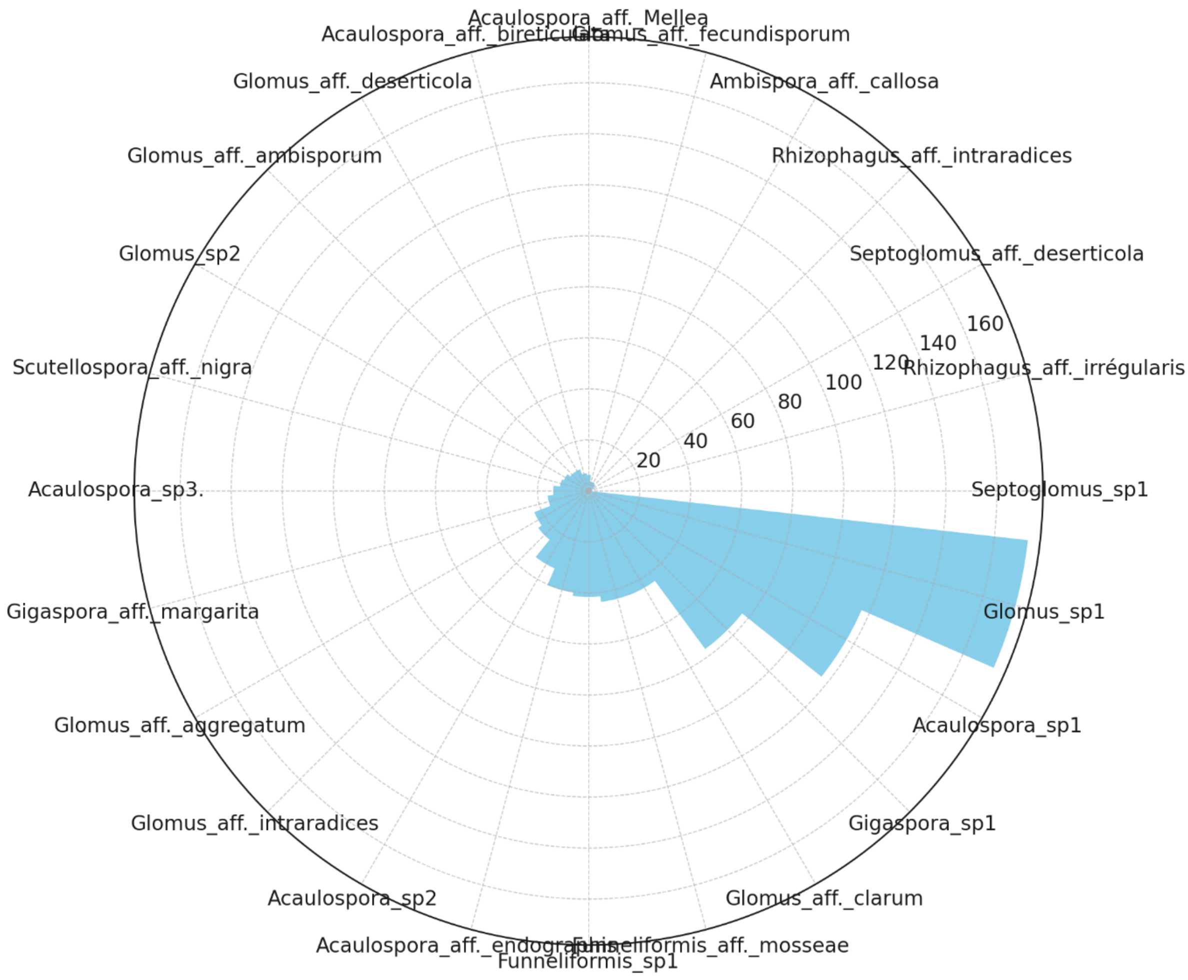


Figure 4: Dominance of AMF species per 100g of soil

5. **Identification of rare or threatened AMF strains**

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.

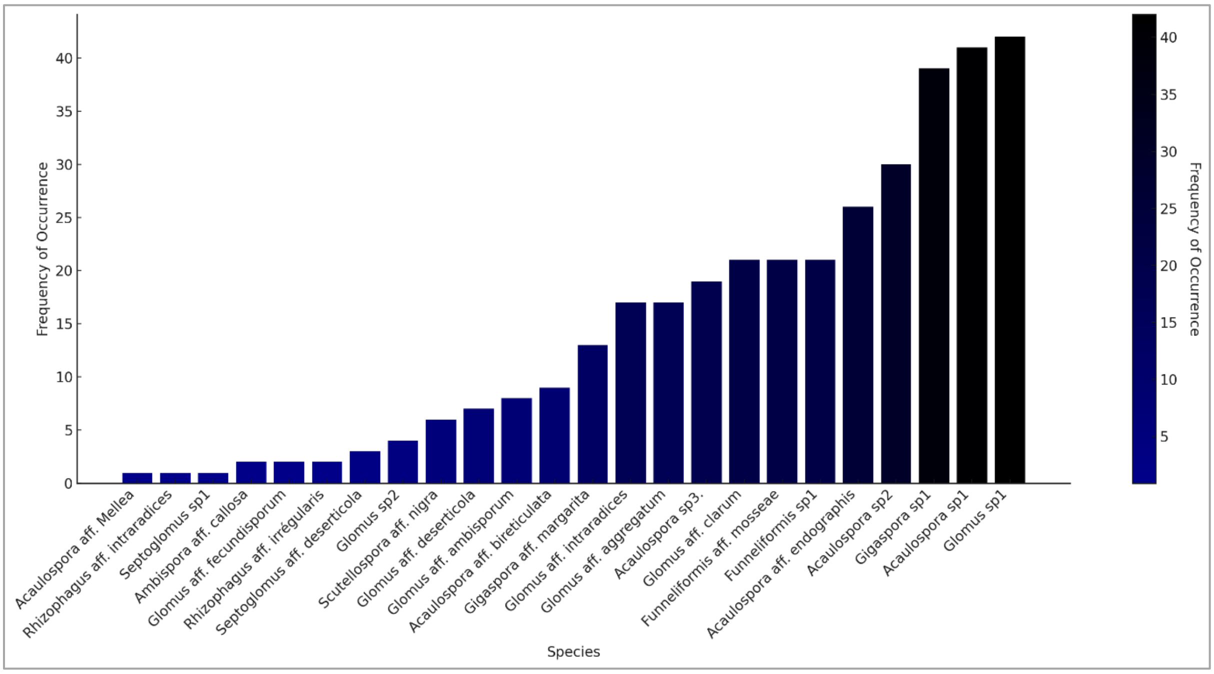


Figure 5: Classification of species according to rarity on the basis of their frequency of appearance in samples

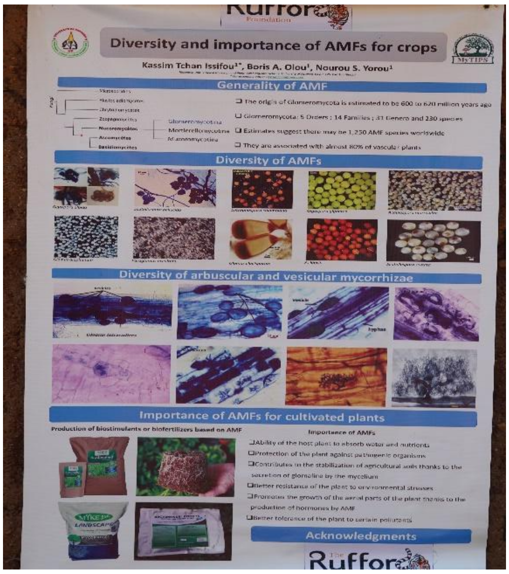
6. **Raising farmers' awareness of best agricultural practices for the conservation of natural AMF habitats.**

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.


Rufford


Rufford


Foundation
Diversity and importance of AMFs for crops
Kassim Tchan Issifou1*, Boris A. Olou1, Nourou S. Yorou1
MyTIPS
Generality of AMF
The origin of Glomeromycota is estimated to be 600 to 620 million years ago
Glomeromycota: 5 Orders ; 14 Families ; 31 Genera and 230 species
Estimates suggest there may be 1,250 AMF species worldwide
They are associated with almost 80% of vascular plants
Glomeromycotina
Mortierellomycotina
Mucoromycotina
Diversity of AMFs
Diversity of arbuscular and vesicular mycorrhizae
vesicle
hyphae
Importance of AMFs for cultivated plants
Production of biostimulants or biofertilizers based on AMF
Importance of AMFs
Ability of the host plant to absorb water and nutrients
Protection of the plant against pathogenic organisms
Contributes to the stabilization of agricultural soils thanks to the secretion of glomaline by the mycelium
Better resistance of the plant to environmental stresses
Promotes the growth of the aerial parts of the plant thanks to the production of hormones by AMF
Better tolerance of the plant to certain pollutants
Acknowledgments
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