

# Exploring the role of indigenous arbuscular mycorrhizal fungi for sustainable soil bio-conservation from Napun Gete Reservoir, Sikka District, Indonesia

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**Abstract.** Jeksen J, Zubair H, Ahmad A, Tenriawaru N. 2025. Exploring the role of indigenous arbuscular mycorrhizal fungi for sustainable soil bio-conservation from Napun Gete Reservoir, Sikka District, Indonesia. *Asian J Agric* 9: 305-315. Soil conservation through biological methods, particularly the use of indigenous Arbuscular Mycorrhizal Fungi (AMF), presents a promising approach to sustainable land management. However, there is limited information on the role of indigenous AMF as agents of soil bio-conservation. This study aimed to explore and evaluate the effectiveness of indigenous AMF from Flores in enhancing soil conservation in the upper region of the Napun Gete Reservoir. A split-plot experimental design was employed, with soil depth (K1: 0-25 cm and K2: 25-50 cm) as the main factor and land unit types (13 units) as subplots. The AMF application followed a randomized block design with five inoculum doses of indigenous Flores AMF: control, 5 g per plant, 10 g per plant, 15 g per plant, and 20 g per plant. The result showed that a total of four AMF genera were identified, including *Glomus* (81.98%), *Acaulospora* (13.29%), *Gigaspora* (4.68%), and *Entrophospora* (0.05%). The highest spore density was recorded in land units with slopes >40%, Andisol soils, and secondary forest land use. Indigenous AMF application significantly affected mycorrhizal, soil, and crop cultivation variables. AMF treatment notably improved soil physical, chemical, and biological properties. This research confirms the potential of indigenous AMF as a viable agent for biological soil conservation, offering a sustainable approach to land management in the study area.

**Keywords:** Andisols, biological conservation techniques, fungal diversity, soil microbiology, sustainable land management

## INTRODUCTION

Soil degradation, a significant environmental concern, is primarily driven by improper land management practices, which accelerate the depletion of soil health and its ability to support sustainable agricultural activities (Nugroho 2024). In the upper Napun Gete Reservoir area, local communities practice land clearing for upland rice cultivation, typically by burning fields 2-3 months before the rainy season. This technique, repeated annually, exposes the soil to environmental forces, leading to significant land degradation. The practice occurs on various terrain types, including steep slopes that often exceed 40%. Preliminary studies have documented erosion rates in the region ranging from 0.05 to 1,719.72 tons per hectare per year, highlighting a wide variation from light to severe erosion. This broad range of erosion intensity indicates the varying degrees of soil degradation, which, if left unaddressed, could severely affect the ecological balance and functionality of the Napun Gete Reservoir in the future.

The challenge of combating soil degradation in such regions requires not only the identification of effective soil conservation methods but also the implementation of strategies that can restore soil health and prevent further degradation. Lal et al. (2021) emphasize the necessity of

rehabilitation and the adoption of resilient and sustainable soil conservation practices to mitigate the long-term impacts of soil erosion. While traditional conservation methods, such as vegetative cover, physical-mechanical techniques, and chemical interventions, have been widely applied (Blanco-Canqui and Lal 2008; Kurniawati et al. 2024), there remains an untapped potential for using biological conservation methods, particularly those involving soil microorganisms (Amallia et al. 2023). Among these, Arbuscular Mycorrhizal Fungi (AMF) have emerged as promising agents for improving soil structure and fertility, yet their application in soil conservation remains relatively underexplored (Jafarpour et al. 2022). Gharemahmudli et al. (2021) suggest that using native microorganisms, specifically those adapted to the local environment, could offer a novel and practical approach to soil and water conservation in regions suffering from degradation.

AMFs have garnered significant attention in recent years due to their ability to enhance soil structure and fertility, particularly in sustainable agricultural systems. Research by Singh et al. (2022) underscores the potential of AMF to mitigate soil degradation by improving soil aggregation and nutrient availability. Lehmann et al. (2017) highlight that mycorrhizal fungi secrete polysaccharides that form organic acids, which bind soil particles into micro

aggregates and stabilize larger soil aggregates. Furthermore, studies by Al-Maliki and Ebreesum (2020) have demonstrated that combining AMF with algae can further enhance soil aggregate formation. Gao et al. (2019) found that AMF-induced glomalin production positively correlates with improved soil aggregate stability. These findings suggest that AMF plays a crucial role in stabilizing the soil environment, supporting microbial communities, and contributing to the sequestration of carbon, all of which are essential for mitigating the effects of soil degradation.

Despite the promising potential of AMF as soil bioconservation agents, research specifically investigating their role in soil degradation and conservation, especially in tropical regions such as Flores Island, remains limited. The application of AMF in soil conservation efforts in Flores is still underexplored, and further studies are needed to assess the presence and effectiveness of indigenous AMF species in the region. This study aimed to investigate the role of indigenous AMF in soil bio-conservation in the upper Napun Gete Reservoir area. Through a comprehensive analysis, this research can provide valuable insights into the presence and potential application of AMF in the region, contributing to a better understanding of their role in sustainable soil management. These findings may also help develop biological soil conservation techniques tailored to the unique environmental conditions of the Napun Gete Reservoir area, offering a model for applying AMF-based conservation strategies in other degraded regions.

## MATERIALS AND METHODS

### Research time and location

The Napun Gete Dam, constructed in 2021, is the first and only dam on Flores Island, located at coordinates 8°32'57"S 122°35'0.6"E, within the administrative boundaries of Ilinmedo Village, Waiblama Sub-district, Sikka District, Flores Island, East Nusa Tenggara Province, Indonesia,

spanning an area of 160 hectares, the dam has a storage capacity of 11.22 million cubic meters (PUPR 2021). The Napun Gete Dam is situated within the Nebe River Basin, which covers a total area of 23,786.17 hectares, and the upstream region of the dam is located in the villages of Natarmage, Tanarawa, and Tua Bao, encompassing an area of 3,223.21 hectares.

The research on the application of CMP (Conservation Management Practices) was conducted in the upstream area of the Napun Gete Dam, specifically in Natarmage Village, Waiblama Sub-district, Sikka District, Indonesia (coordinates 8°36'14.13"S 122°34'3.93"E), at an elevation of 391 meters above sea level, from September 2023 to January 2024. The study area experiences an average annual temperature of 21°C and an annual rainfall of 1,500 mm. The soil in this region is classified as Andisols, with a clayey texture consisting of 28% sand, 34% silt, and 38% clay.

### AMF exploration

Exploration activities were carried out in the upstream area of the Napun Gete Dam in February 2023. The exploration followed an experimental design using a split-plot design, where the main plots represented the soil depth factor, which consisted of two depth ranges: 0-25 cm and 25-50 cm. The subplots represented the land unit factor, which comprised 13 land units. According to land use mapping (Figure 1.A) in the upstream area of the Napun Gete Dam, three types of land use were identified: secondary forest, mixed gardens, and shrubland. Based on slope mapping (Figure 1.B) in the study area, slopes were categorized into three classes: 0-25%, 25-40%, and >40%. Soil type mapping (Figure 3) revealed two primary soil types in the area: Andisols and Alfisols. Overlaying the three maps above resulted in 14 land unit combinations (Figure 2). However, one land unit was categorized as a settlement area and was eliminated, leaving 13 land units for further analysis.

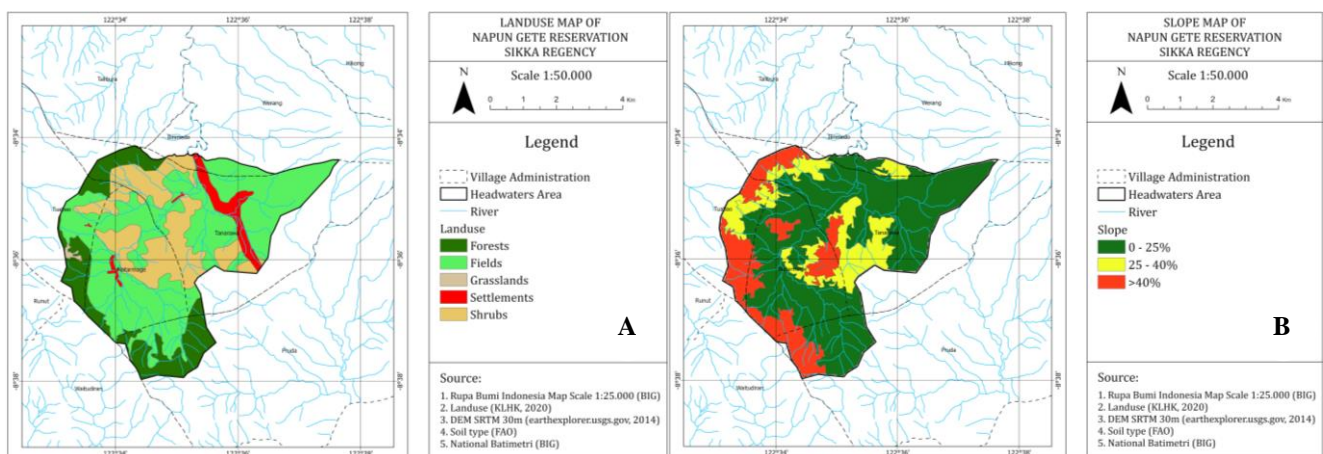


Figure 1. A. Sampling land map, B. Slope map

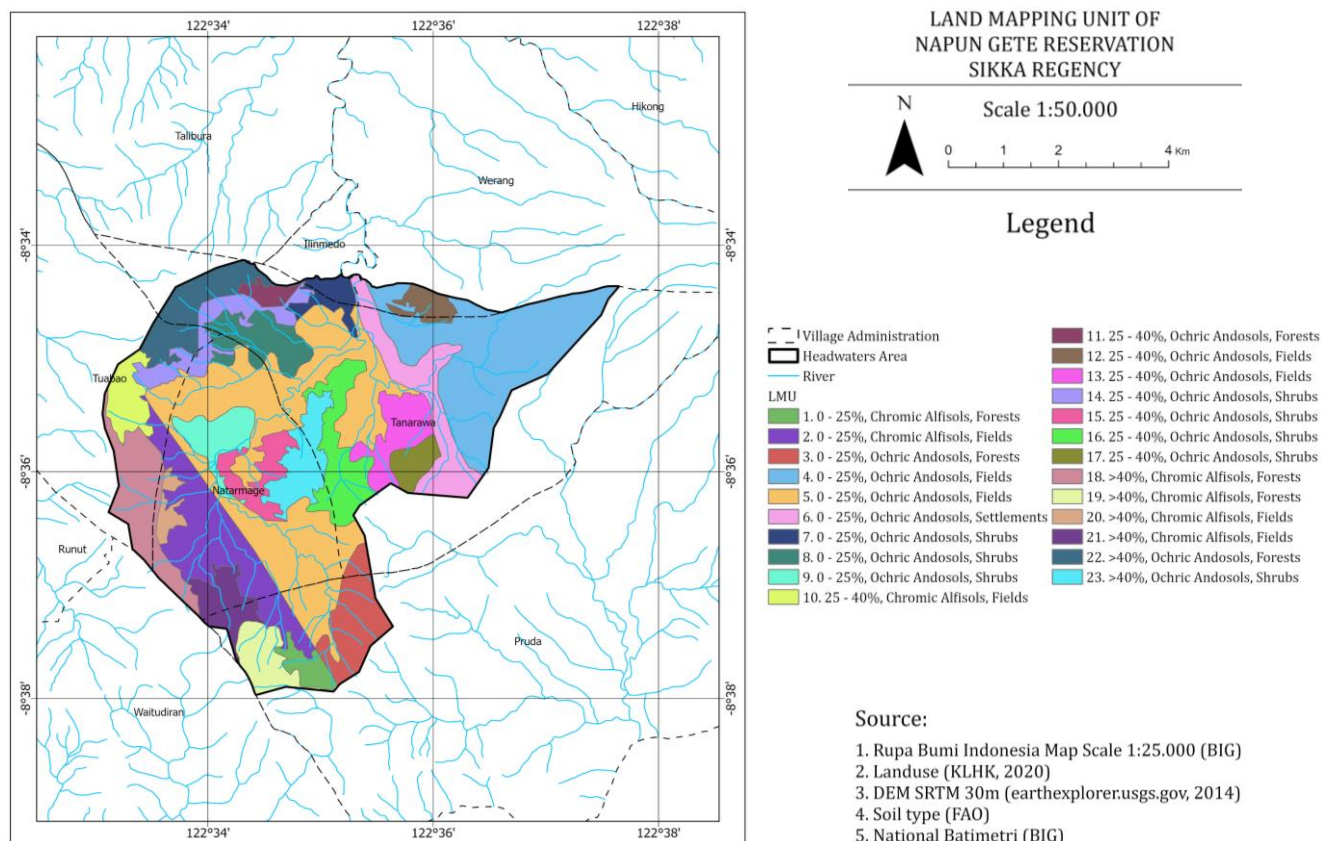


Figure 2. Land unit map

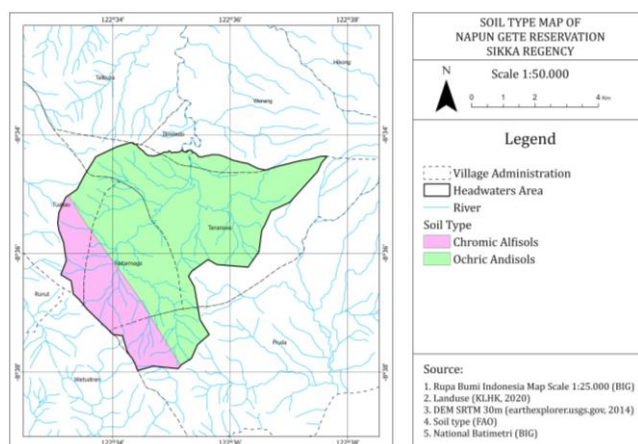


Figure 3. Soil type map

The details of the 13 land units were as follows: U1, located on a 0-25% slope, features Alfisols and secondary forest. U2, with the same slope range, also had Alfisols but was characterized by a mixed garden. U3, located on a 0-25% slope, had Andisols and was covered by secondary forest. U4, within the same slope range, also had Andisols and a mixed garden. U6, found on a 0-25% slope, comprised Andisols and shrubland. Moving to a 25-40% slope, U7 features Alfisols and a mixed garden, while U8, with a similar slope, has Andisols and a secondary forest. U9, also on a 25-40% slope, consists of Andisols and a mixed garden.

Within this slope range, U10 contained Andisols and shrubland. U11, located on a slope greater than 40%, had Alfisols and a secondary forest. Alfisols and mixed gardens were found in U12, which also had a slope over 40%. U13, on a slope greater than 40%, had Andisols and secondary forests, while U14, also with a slope greater than 40%, consisted of Andisols and shrubland.

Soil sampling was repeated three times at each land unit, resulting in 78 soil samples (13 units × 2 depths × 3 replications). A 500 g soil sample was taken from the rhizosphere of each unit. Next, to identify the species and abundance of soil spores (AMF), spores were separated from the soil using sieving and centrifugation methods, as described by Brundrett et al. (1996). After isolation, spores were identified using microscopic methods, focusing on morphological characteristics such as shape, color, and ornamentation. The identification of spores was based on comparisons with spore standards from Brundrett et al. (1996), INVAM (2023a), and Pérez and Schenck (1990). This process allowed for determining the types and quantities of AMF spores in the soil samples, providing valuable data on the distribution and abundance of these vital soil microorganisms in the region.

**Preparation of inoculum for indigenous Flores AMF**

AMF (Colonization Mycorrhizal Inoculum) indigenous Flores was produced on sterile fine sand media. The planting medium was mineral soil once used for cultivating corn in dry fields that was sterilized using an autoclave, and the

media were allowed to cool for 24 hours before use. The inoculum preparation took place in pots using 1 kg of sterile soil, with corn plants (INVAM 2023b) serving as the host plant. This process was specifically targeted towards the most dominant AMF species identified in the exploration activities conducted in the upstream area of the Napun Gete reservoir, namely the *Glomus* genus. The number of pots used was determined based on the research requirements, with two pots containing 100 spores each. The planting media was regularly watered throughout its growth period to maintain moisture. However, at six weeks of plant growth, watering was halted for two weeks to enhance spore growth. Fertilization was carried out using a phosphorus-free Yoshida solution. The fertilizer dosages were as follows: 10 mL of solution during the first week, 20 mL each week during weeks 2-3, and 20 mL twice a week from 4 to 6 weeks.

At eight weeks or two months of age, AMF inoculum was ready to be harvested. Harvesting the inoculum involved cutting the host plant at the base of the stem. Subsequently, the corn plant roots were chopped into small 1 cm pieces, then reintroduced into the pot and mixed thoroughly. This process yielded the AMF inoculum, which was then ready for use (Brundrett et al. 1996). The careful management of soil sterilization, watering schedules, fertilization, and the timing of inoculum harvest ensured the optimal growth of the mycorrhizal spores, highlighting the importance of controlled environmental conditions for successful AMF production.

#### AMF application experimental design

The experiment employed a randomized block design with a single factor pattern consisting of five inoculum doses of indigenous Arbuscular Mycorrhizal Fungi (AMF) from Flores (M): (i) control, with no AMF inoculum added (M0), (ii) 5 g per plant of AMF inoculum (M1), (iii) 10 g per plant of AMF inoculum (M2), (iv) 15 g per plant of AMF inoculum (M3), and (v) 20 g per plant of AMF inoculum (M4). Each treatment was replicated four times, resulting in 20 experimental units. The experiment utilized pots measuring 24 cm in height and 30 cm in diameter, with a soil capacity of 8 kg per pot. The experimental area covered 8 × 6 m. The indicator plant used was the Ciharang variety of upland rice, with seven seeds planted per pot. The application of AMF inoculum was performed simultaneously with the planting of seeds. An approximately 5-10 cm deep hole was made using a wooden stick, and AMF inoculum was manually placed into the soil. The hole was then partially covered with soil, and the rice seeds were planted in the same hole, followed by full coverage of the hole with soil.

Mineral fertilization was applied at 50% of the recommended standard dose, which included urea at 100 kg ha<sup>-1</sup>. Fertilization was carried out in three stages: 40% of the urea dose (0.16 g per plant) was applied at planting, 30% (0.12 g per plant) at 15 Days After Sowing (DAS), and 30% (0.12 g per plant) at 30 DAS. Additionally, SP-36 was applied at 50 kg ha<sup>-1</sup> (0.12 g per plant) and KCl at 50 kg ha<sup>-1</sup> (0.12 g per plant) at planting time. The rice plants were harvested 126 Days After Planting (DAP). Pest control

and weed management were carried out throughout the plant's growth period. The design and treatment aimed to investigate the effect of various AMF inoculum doses on the growth and yield of upland rice.

#### Soil properties analysis

Soil properties were evaluated at the beginning and end of the experiment by collecting topsoil samples from a depth of 0 to 20 cm. The soil samples were air-dried, homogenized, and passed through a 2 mm sieve. Soil pH was determined using the electrometric method (pH in H<sub>2</sub>O). Organic carbon content was measured using the Walkley and Black (1934) method. Total nitrogen was determined by the Kjeldahl (ACIAR 1990) method, while available nitrogen (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>) was assessed using the potassium chloride (KCl) extraction (Lisle et al. 1991) method. The available phosphorus content was quantified using the Olsen (Olsen et al. 1954) method, and the total phosphorus and potassium content was determined using the 25% HCl extraction (Sudjadi et al. 1971) method. All soil property determinations followed the protocols Horwitz and International (2000) outlined.

This comprehensive analysis of soil characteristics provided essential insights into the soil's nutrient status and potential for supporting plant growth under different experimental treatments.

#### AMF properties

The infectivity of Arbuscular Mycorrhizal Fungi (AMF) was assessed using a staining method based on the protocol established by Brundrett et al. (1996). This method involved using specific stains that allow for the clear visualization of fungal structures within plant roots, thereby facilitating the evaluation of the degree of fungal colonization.

Additionally, the sporulation of arbuscular mycorrhizal fungi was determined using the wet-sieving method, following the guidelines set by Brundrett et al. (1996). The ability of AMF to sporulate efficiently was crucial for their long-term survival and colonization success. The UV-Vis spectrophotometric method was employed to quantify the presence of glomalin, a glycoprotein produced by AMF that plays a significant role in soil aggregation and stability, as Wright and Upadhyaya (1998) described.

#### Statistical analysis

All the results of exploration activities, particularly the data on the abundance of Arbuscular Mycorrhizal Fungi (AMF), the effects of indigenous flores arbuscular mycorrhizal fungi on AMF, were evaluated through Analysis of Variance (ANOVA) with a split-plot design to assess the number of spores present. Tukey's post-hoc test was conducted at a significance level 0.05 to determine the differences between treatments. Additionally, correlation analysis were performed to explore the relationships between various parameters, such as spore abundance, soil properties, and plant performance. The statistical analysis was carried out using the IBM SPSS software package, version 23.0, which offers robust data analysis and hypothesis testing tools.

## RESULTS AND DISCUSSION

### Exploration of AMF species at Napun Gete Reservoir Watershed

The exploration of AMF species within the Napun Gete Dam's upstream area revealed several genera, including *Glomus*, *Acaulospora*, *Gigaspora*, and *Entrophospora* (Figure 4). Among these, the *Glomus* genus was the most dominant, accounting for 81.98% of the total AMF population, followed by *Acaulospora* at 13.29%, *Gigaspora* at 4.68%, and *Entrophospora* at a minimal 0.05%. These findings highlight that *Glomus* species exhibited a broad distribution across the entire upstream area of the Napun Gete Reservoir, consistently detected in soil samples collected from 0-25 and 25-50 cm depths. In contrast, *Acaulospora*, *Gigaspora*, and *Entrophospora* were often absent in some collected samples. This observation aligns with the findings of Herawati et al. (2024), who noted that in dryland ecosystems, *Glomus*, *Acaulospora*, and *Gigaspora* dominate AMF populations. In contrast, *Entrophospora* and *Scutelospora* are found in much smaller quantities.

Furthermore, it was also suggested that *Glomus*, *Acaulospora*, and *Gigaspora* dominate in dry environments due to their superior adaptive capacity (Sefrila et al. 2023). These genera were endomycorrhizal fungi well-suited to survive in arid conditions, exhibiting greater spore germination rates and resilience than *Scutelospora* and *Entrophospora*. This adaptability likely contributes to *Glomus*'s persistence and widespread occurrence in the Napun Gete watershed, indicating its ecological significance in maintaining soil health and supporting plant growth in this environment. The ability of these fungi to thrive in challenging conditions further emphasizes the importance of their role in ecosystem functioning, particularly in regions with variable and dry climates (Herawati et al. 2021).

These findings indicate that the abundance of AMF spores was significantly higher in secondary forests than in other land uses, primarily due to vegetation regeneration. The regrowth of plants in secondary forests increases the availability of organic carbon. It restores host plants for mycorrhizal fungi, which fosters the growth and reproduction of AMF spores. This aligns with previous research by Reyes et al. (2019), which found that soil characteristics, such as organic matter content, pH, aluminum concentration, cation exchange capacity, and calcium have strong relationships with abundant *glomerospora* and AMF species

composition. The presence of an organic litter layer in secondary forests further supports the growth of mycorrhizal fungi by providing a steady source of energy and nutrients. This is consistent with findings by Chen et al. (2023), who noted that slope gradients affect AMF spore abundance, with lower spore counts generally found in steeper slopes due to less stable soil conditions. However, they also observed that other factors, such as soil stability, water availability, and nutrient levels, in combination with vegetation cover, are crucial in determining AMF populations and activity across varying slope gradients.

Regarding soil type, Andisols exhibited higher fungal spore counts compared to Alfisols. This can be attributed to the higher organic matter content in Andisols, which generally supports greater fertility and fungal activity. The results of this study demonstrate that soil properties, such as organic matter content, pH, and phosphorus fixation, play a crucial role in shaping the abundance and community composition of AMF spores. Furthermore, while slope gradients can affect fungal abundance, various factors-including vegetation cover and soil stability-determine the extent of AMF proliferation. However, Andisols also exhibit higher phosphate fixation rates, which may limit phosphorus availability to both plants and mycorrhizal fungi. Alfisols, while having lower organic matter content and fertility, showed different patterns in spore abundance, indicating that soil chemical properties significantly influence fungal populations. Secondary forests, through their ability to regenerate vegetation and provide an organic layer of litter (Wiratmaja et al. 2024), create favorable conditions for the growth of mycorrhizal fungi.

### AMF application

#### AMF abundance

An Analysis of Variance (ANOVA) showed a highly significant effect on AMF abundance (Figure 5). The highest spore abundance reached 110 spores per 10 g of soil in the treatment with indigenous AMF from Flores at 20 g pot<sup>-1</sup> (M4), 79.77% higher than the control treatment without AMF (M0). The treatment without AMF (M0) significantly differed from the other AMF treatments. Further statistical tests on AMF spore abundance are shown in Figure 6. These findings underscore the potential of indigenous AMF strains in significantly enhancing spore population density and, by extension, their ecological function in promoting plant growth and soil health.



**Figure 4.** AMF spores identified under a binocular microscope (400X magnification): A. *Acaulospora foveata* Trappe & Janos, B. *Acaulospora undulata* Sieverd., C. *Glomus mosseae* (T.H.Nicolson & Gerd.) Gerd. & Trappe, D. *Glomus etunicatum* W.N.Becker & Gerd., E. *Gigaspora margarita* W.N.Becker & I.R.Hall, F. *Entrophospora infrequens* (I.R.Hall) R.N.Ames & R.W.Schneid.

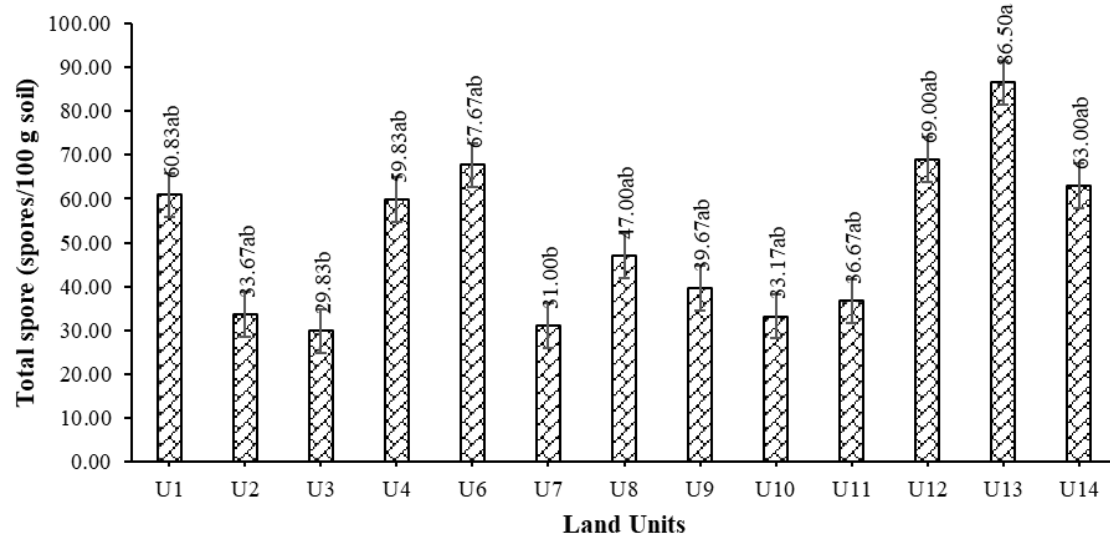


Figure 5. Effect of land unit on the number of AMF spores

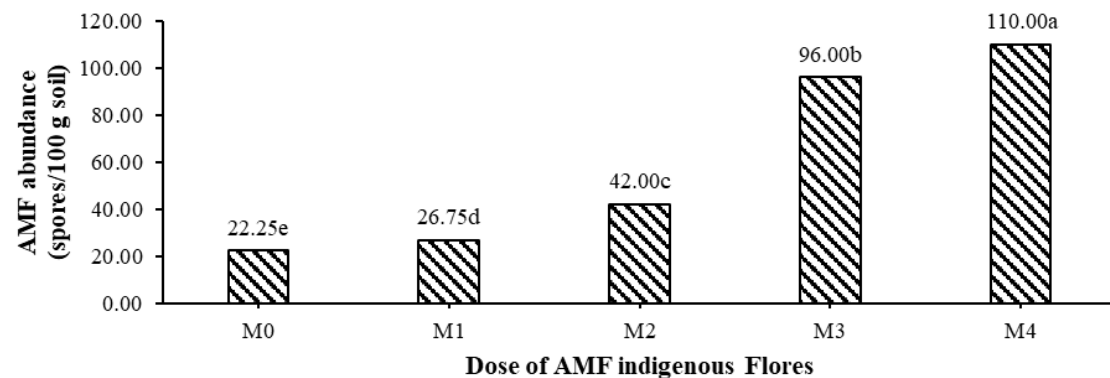
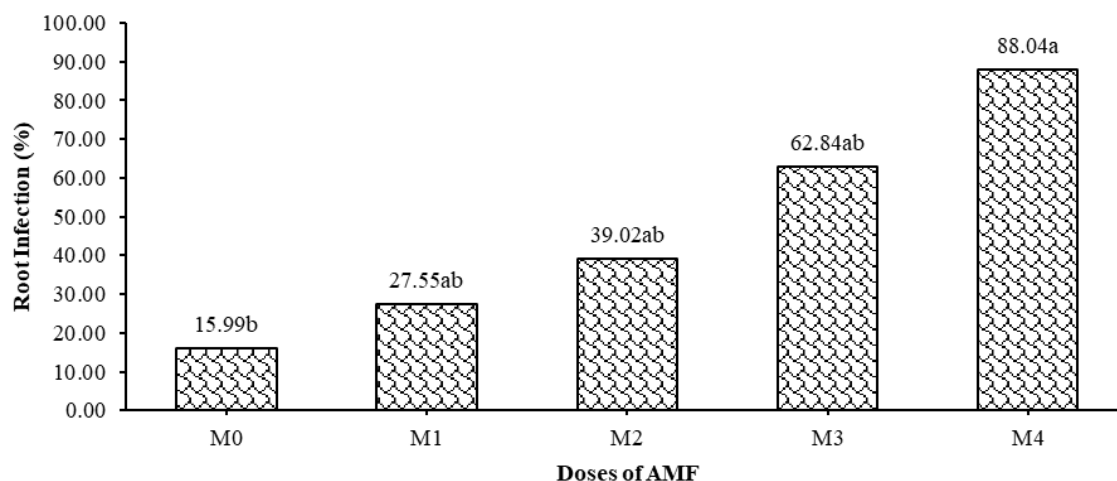


Figure 6. Effect of AMF on abundance

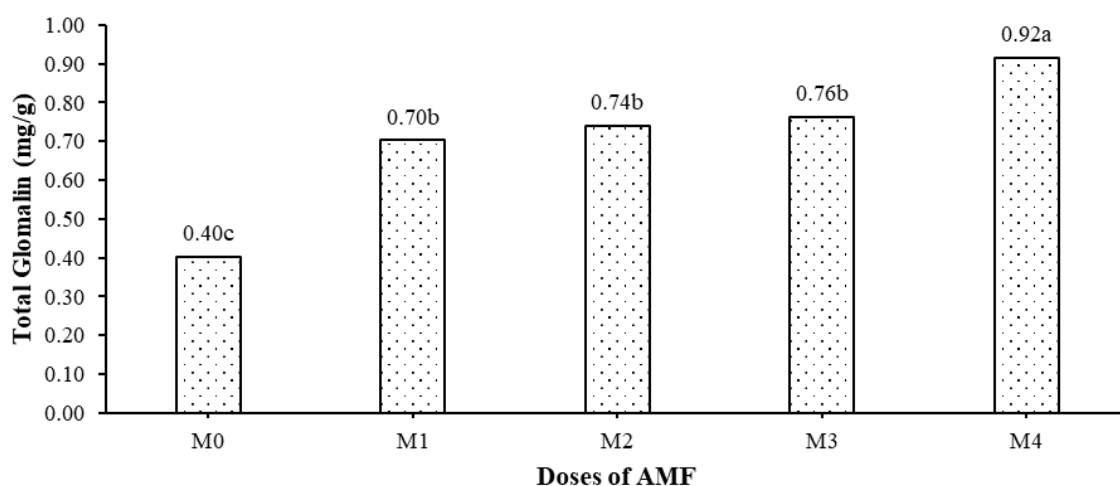
The abundance of Arbuscular Mycorrhizal Fungi (AMF) is influenced by three main factors: host plant availability, climate, and soil nutrients. First, compatible host plants are crucial, as they provide carbohydrates that stimulate AMF spore formation. Certain crops like rice, maize, and legumes support AMF, making plant selection an important strategy to enhance fungal activity in agroecosystems. Second, environmental conditions such as humidity and temperature also play a role. High humidity and moderate temperatures promote AMF growth and sporulation, while drought and extreme heat reduce it (Mohamed et al. 2023). The study area's average humidity of around 85% suggests favorable conditions for AMF development. Third, soil nutrient content—especially phosphorus—affects AMF presence. AMF tends to thrive in nutrient-poor soils where plants depend more on symbiosis to access nutrients. Soil analysis from the study area reveals low nitrogen, phosphorus, and potassium levels, with slightly acidic pH, indicating a low-fertility environment suitable for AMF proliferation. The combination of appropriate host plants, supportive climate, and nutrient-poor soil makes the study area a promising habitat for native AMF species (Dierks et al. 2021).

#### Percentage of root infection by AMF

The percentage of root infection by Arbuscular Mycorrhizal Fungi (AMF) is a crucial indicator for evaluating the effectiveness of the mutualistic symbiosis between mycorrhizal fungi and plant roots. The results exhibited that the percentage of root infection by indigenous AMF from Flores showed a highly significant effect. The highest infection rate reached 88.04% in the treatment with 20 g of indigenous AMF per pot (M4), an increase of 81.84% compared to the untreated control (M0). The treatment without AMF (M0) significantly differed from the 20 g per pot AMF treatment (M4), while other treatments did not show significant differences. The results of the post-hoc test on AMF's role in root infection percentage are presented in Figure 7. In the M0 treatment, infection was observed, indicating the natural presence of AMF at the study site. Despite soil sterilization at 121°C, AMF survived, highlighting its resilience.



**Figure 7.** Effect of AMF on the percentage of root infection



**Figure 8.** Effect of AMF on total glomalin

Root infection by Arbuscular Mycorrhizal Fungi (AMF) occurs through the penetration of fungal hyphae into the cortical root tissue. The hyphae form specialized structures known as arbuscules and vesicles within the cortical cells. Arbuscules function as nutrient exchange points between fungi and plants, while vesicles serve as nutrient reserves (Tedersoo et al. 2020). This process provides mutual benefits: the plant gains access to additional nutrients, especially phosphorus, while the fungi receive carbon sources from the plant's photosynthesis. According to Dowarah et al. (2022), the percentage of root infection by AMF is significantly influenced by several factors, including plant species, soil nutrient availability, environmental conditions, and organic matter and fertilizers. Research by Paymaneh et al. (2023) and Fall et al. (2022) indicates that combining organic fertilizers and AMF inoculation can enhance root infection percentage while improving soil physical, chemical, and biological properties.

#### Total glomalin

Glomalin is a soil protein that AMF hyphae produce and is a vital component of soil organic matter. One of the primary roles of glomalin is maintaining soil aggregate stability. Stable aggregates are crucial for preventing erosion and supporting root systems. Based on the results of the ANOVA, total glomalin showed a significant effect. The highest value was recorded at 0.92 mg g<sup>-1</sup> soil in the treatment with 20 g of indigenous AMF per pot (M4), representing an increase of 56.10% compared to the untreated control (M0). The treatment without AMF (M0) significantly differed from the other AMF treatments. The post-hoc test results on the role of AMF in total glomalin are presented in Figure 8.

This aggregate stability enhances the soil's ability to retain water, prevents nutrient leaching, and provides optimal conditions for root growth. Matos et al. (2021) highlighted that glomalin plays a significant role in improving soil quality by enhancing soil structure, increasing organic matter content, reducing fertility decline, and boosting microbial activity, thus aiding in the restoration of heavy metal-contaminated soils.

**Table 1.** Soil analysis results after treatment

AMF	pH	Organic-C (%)	Total-N (%)	Available N		Total-P (mg/100 g)	Available-P (ppm)	Total-K (mg/100 g)
				NO <sub>3</sub> <sup>-</sup> (ppm)	NH <sub>4</sub> <sup>+</sup> (ppm)			
M0	5.58 <sup>b</sup>	1.63 <sup>e</sup>	0.16 <sup>e</sup>	1.34 <sup>c</sup>	1.94 <sup>b</sup>	26.12 <sup>e</sup>	8.79 <sup>b</sup>	17.14 <sup>c</sup>
M1	5.43 <sup>c</sup>	2.06 <sup>d</sup>	0.36 <sup>c</sup>	2.58 <sup>ab</sup>	4.0 <sup>ab</sup>	31.47 <sup>d</sup>	9.31 <sup>ab</sup>	20.92 <sup>ab</sup>
M2	5.55 <sup>bc</sup>	2.63 <sup>b</sup>	0.30 <sup>d</sup>	3.48 <sup>a</sup>	3.06 <sup>ab</sup>	33.35 <sup>c</sup>	10.17 <sup>ab</sup>	20.24 <sup>b</sup>
M3	5.81 <sup>a</sup>	2.56 <sup>c</sup>	0.39 <sup>b</sup>	2.45 <sup>b</sup>	4.02 <sup>ab</sup>	34.70 <sup>b</sup>	10.65 <sup>ab</sup>	22.21 <sup>a</sup>
M4	5.20 <sup>d</sup>	2.79 <sup>a</sup>	0.45 <sup>a</sup>	3.31 <sup>ab</sup>	5.23 <sup>a</sup>	37.17 <sup>a</sup>	11.51 <sup>a</sup>	21.03 <sup>ab</sup>

Note: Treatment means followed by the same letter are not significantly different

Glomalin production is influenced by several factors, including soil type and conditions, agricultural practices, and the presence of AMF (Arbuscular Mycorrhizal Fungi). Soils with low disturbance levels, such as organic farming systems, tend to have higher glomalin levels. In contrast, intensive soil cultivation can reduce AMF populations, lowering glomalin production. Adding organic fertilizers such as compost and biochar has also been reported to increase the total glomalin content in the soil. In agricultural soils, the glomalin content varies between 0.30-0.70 mg g<sup>-1</sup> of soil (Hossain 2021).

Glomalin, produced by AMF, is significant in physical, chemical, and biological soil conservation efforts. Singh et al. (2022) stated that glomalin plays a role in improving the soil's physical, chemical, and biological properties, helping to rehabilitate land degraded by activities such as mining, erosion, intensive farming, excessive salinity, deforestation, and grazing.

### Soil characteristics

Based on the laboratory analysis results presented in Table 1, it was found that the chemical content of the soil showed different variations across the treatments. The post hoc test results indicated significant differences between the control treatment (M0) and the other treatments. The M0 treatment significantly differed from all other treatments for all the tested soil chemical properties except for the soil pH variable. Additionally, an increase was observed when comparing the initial soil analysis results with the post-treatment soil analysis results in Table 1. This suggests that the indigenous AMF treatment from Flores applied in the experiment effectively improved the soil's chemical properties.

As a result of the soil acidity value, the best treatment was found in the indigenous AMF treatment of 15 g pot<sup>-1</sup> (M3), which showed the highest pH result, namely 5.81. Based on the soil-chemical property evaluation criteria the Soil Research Institute set, this value (M3) was slightly acidic, while other treatments fall into the acidic category. The results showed that soil acidity tended to decrease, and only the M3 treatment showed consistent results compared to before and after the indigenous Flores AMF treatment. This is because this study used mineral fertilizers such as urea, SP-36, and KCl, which were applied to all treatments. Based on the research by Robertson and Groffman (2024), when urea fertilizer is applied to the soil, it undergoes hydrolysis by the enzyme urease, producing ammonium (NH<sub>4</sub><sup>+</sup>) and carbon dioxide (CO<sub>2</sub>). This process can temporarily increase soil pH due to the formation of

alkaline ammonium ions. Potassium ions can replace hydrogen ions in the soil colloid complex, increasing the H concentration<sup>+</sup> in the soil solution and making the soil pH more acidic (Kurniawan et al. 2023).

The application of indigenous Flores AMF treatment on soil organic carbon showed significant results. The best treatment was found in the indigenous Flores AMF treatment of 20 g pot<sup>-1</sup> (M4), which showed the highest soil C-organic result, 2.79%. According to the Soil Research Institute, the soil-chemical property evaluation standards, M4 is classified in the moderate category. Meanwhile, all other treatments, except for M0, are also categorized as moderate, while M0 is categorized as low (BPT 2005), though they have varying percentages of C-organic. The results of further tests on the role of AMF in soil C-organic are presented in Table 1. According to Basiru and Hijri (2024), AMF plays an essential role in soil organic carbon dynamics and uniquely transfers carbon (C) obtained from its host plants into the soil. This carbon can be labile organic matter (easily decomposed) or recalcitrant organic matter (hard to decompose). This process results in different impacts on soil organic carbon reserves. Labile materials tend to accelerate the carbon cycle because they decompose quickly, while recalcitrant materials contribute to long-term carbon storage, thus increasing carbon stability in the soil. According to Ren et al. (2021), AMF inoculation can be an effective agricultural practice in increasing soil C-organic.

As the percentage of infection and AMF abundance increases, the nitrogen content in the soil also increases, both in the form of total N and nitrate (NO<sub>3</sub><sup>-</sup>) and ammonium (NH<sub>4</sub><sup>+</sup>) uptake. The best results for total N, NO<sub>3</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup> content from indigenous Flores AMF treatment were found in the 20 g pot<sup>-1</sup> treatment (M4), with results of 0.45%, 3.31 ppm, and 5.23 ppm, respectively. According to the soil-chemical property evaluation criteria by the Soil Research Institute, the total N content in all treatments, except for M0, is moderate (BPT 2005). However, the % total N varies between treatments. When comparing the best treatment (M4) to the untreated treatment (M0), there was an increase of 65.36, 59.67, and 62.87%, respectively. The results of further tests on the role of AMF in total N, NO<sub>3</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup> content are presented in Table 1. The increase in nitrogen content is due to AMF forming an external hyphal network that extends deep into the soil, outside the root zone of plants. This network helps absorb nitrogen from sources complex for plant roots to access, such as organic compounds bound to soil particles. AMF hyphae increase the soil contact area, enhancing nitrogen uptake (Suwardi et al. 2023). Nitrogen increases not only

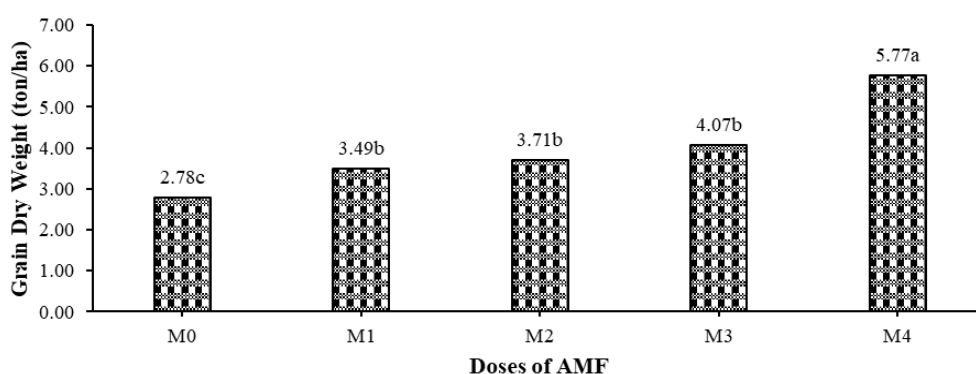
through hyphae but also through the mineralization of organic nitrogen. AMF can interact with other soil microorganisms to accelerate the decomposition of organic material. In this process, organic nitrogen compounds are converted into inorganic forms such as ammonium ( $\text{NH}_4^+$ ) or nitrate ( $\text{NO}_3^-$ ), which plants can absorb. The stimulation of microbial activity by AMF can encourage soil microorganisms involved in the nitrogen cycle, such as free-living nitrogen-fixing bacteria (*Azotobacter*) or nitrifying bacteria. The interaction between AMF and soil microbes accelerates processes that support the increased availability of total N.

Furthermore, according to Qiu et al. (2022), the presence of AMF significantly reduces nitrogen (N) and phosphorus (P) losses in the soil. According to Mulyadi and Jiang (2023), the combined application of AMF and nitrogen (N) fertilizers has benefitted rice farming systems significantly. This combination improves soil physicochemical properties, such as C-organic content and total glomalin, and positively impacts rice's plant growth, crop yields, and nitrogen absorption efficiency. In line with the main role of AMF, which is to increase phosphorus (P) content in the soil, this study also showed an increase in total P and available P content after treatment. Overall, all treatments showed an increase in total P and available P content, with a significant difference between M0 and all treatments. The highest increase in total P and available P was observed in the M4 treatment, with values of 37.17 mg/100 g and 11.51 ppm, respectively. According to the soil-chemical property evaluation criteria set by the Soil Research Institute, total P content in all treatments is categorized as moderate, and available P content increased from low to moderate levels before and after the treatment (BPT 2005). This is due to AMF's ability to produce phosphatase enzymes that can release P-bound to soil minerals or organic materials that are difficult for plants to access, making it available to plants. Furthermore, the increase in P occurs due to the expansion of the hyphal network, as AMF has external hyphal structures that can reach soil areas outside the plant root zone (rhizosphere). These hyphae expand the plant's capacity to absorb phosphorus from the soil, especially from forms of phosphorus that are difficult for plant roots to access directly (Etesami et al. 2021). Research on maize plants has shown that AMF inoculation increases the uptake of phosphorus, nitrogen, and other micronutrients (Qin et al. 2022; Peng et al. 2023).

### Effect of AMF on upland rice plant yield

Based on the ANOVA results, the dry weight of grain ha<sup>-1</sup> showed a very significant effect. The highest yield reached 5.77 tonnes in the 20 g pot<sup>-1</sup> AMF native Flores treatment (M4), or an increase of 51.86% compared to no treatment (M0). Figure 9 shows that the treatment without AMF (M0) significantly differed from the other AMF treatments. The results of tests on the role of AMF on total glomalin are presented in Figure 9.

The accumulation of symbiosis between AMF (Arbuscular Mycorrhizal Fungi) and plants increases plant production. This is in line with the research findings of Ishaq et al. (2021) that plants inoculated with AMF significantly increase soil-available phosphorus (P), plant growth, and corn crop yield compared to non-inoculated plants, as supported by the study of Putra et al. (2020) which stated that AMF can improve the growth and yield of chili plants. AMF plays a vital role in enhancing plant growth and yield through several mechanisms, namely, improving nutrient absorption through AMF hyphae that can extend the root absorption area. This allows the plant to absorb all macro and micronutrients, especially phosphorus (P), which is typically hard to obtain in dry land. Second, improving soil structure via glomalin production contributes to soil aggregates' stability. A better soil structure increases the soil's capacity to retain water and maintain moisture, which is crucial in dryland rice fields that often face water shortages. Third, by stimulating growth hormones, AMF affects plant metabolism, which can ultimately boost the production of hormones such as auxins, cytokinins, and gibberellins in the host plant. These hormones play a role in stimulating root and vegetative part growth, thus improving productivity. Fourth, through increasing the organic carbon content and soil microbial activity, AMF symbiosis with plants can enhance the organic carbon content in the soil through contributions from root and root exudate-derived organic matter. Soil microorganism activity also increases due to the abundance of carbon available as an energy source for their growth, supporting soil fertility and plant health. Additionally, according to the research findings of Zou et al. (2021), it is said that AMF can regulate physiological and molecular responses to drought stress and counteract oxidative damage caused by drought by enhancing antioxidant defense systems.



**Figure 9.** Effect of AMF on Grain Dry Weight (GDW)

In conclusion, in this study, a total of four genera, namely *Glomus* (81.98%), *Acaulospora* (13.29%), *Gigaspora* (4.68%), and *Entrophospora* (0.05%), were explored in the upstream area of the Napun Gete Dam. The highest number of spores was found in land units with a slope greater than 40%, andisol soil types, and secondary forest land use. In the application phase, indigenous AMF from Flores contributed significantly to the results among all the observed variables, including mycorrhiza, soil, and crop cultivation. Based on the statistical result, AMF can improve soil's physical, chemical, and biological properties. Therefore, it can be concluded that this study has successfully discovered the biological soil conservation approach model by utilizing indigenous AMF as a soil bio-conservation agent. Further research is needed to develop a large-scale indigenous AMF biological fertilizer product for broader soil conservation efforts.

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