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From nursery to field: context-dependent benefits of native mycorrhizal inoculation on Khaya madagascariensis seedlings in degraded highland ecosystems of Madagascar

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From nursery to field: context-dependent benefits of native mycorrhizal inoculation on *Khaya madagascariensis* seedlings in degraded highland ecosystems of Madagascar

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Abstract

Restoring vegetation on degraded tropical slopes remains a major challenge due to strong edaphic constraints limiting plant establishment. Although arbuscular mycorrhizal fungi (AMF) are known to enhance plant performance, their effectiveness under field conditions and across contrasting environments remains insufficiently understood. In this study, we investigated how site-specific soil properties and native AMF communities influence the performance of *Khaya madagascariensis*, an endemic tree species used in ecological restoration, from nursery to field conditions across two degraded sites in Madagascar (Boeny and Betsiboka).

Soil analyses revealed marked edaphic contrasts between sites, associated with significant differences in AMF community structure. Betsiboka exhibited higher spore density (5 900 ±

601 vs 3 674 ± 537 spores per 100 g soil), diversity (H' = 1.545 vs 1.107), and evenness (0.862 vs 0.688). Under nursery conditions, AMF inoculation significantly enhanced seedling performance, increasing shoot biomass ($p < 0.0001$), root biomass ($p < 0.05–0.01$), and mycorrhizal colonization rates (from ~20–23% to ~52–59%, $p < 0.01$). After transplantation, inoculated seedlings generally showed improved performance; however, the magnitude and direction of this effect varied between sites. A stronger positive response was observed at Boeny, whereas at Betsiboka the effect was weaker and, in some cases, associated with reduced survival.

These results demonstrate that AMF-mediated benefits are not universal but strongly context-dependent. The stronger response under more limiting conditions suggests that AMF symbiosis plays a key role in alleviating environmental constraints. This study highlights the importance of site-adapted microbial communities and supports the integration of plant–soil–microbe interactions into restoration strategies for degraded tropical ecosystems.

Key words: AMF spores, Restoration, *Khaya madagascariensis,* Inoculation, Madagascar

Introduction

Degraded mountainous ecosystems represent major challenges for ecological restoration worldwide, particularly in tropical regions where intense land-use pressure and recurrent disturbances have severely altered natural vegetation dynamics (Lamb et al., 2005; Holl, 2017). In Madagascar, bushfires, deforestation and increasing anthropogenic pressure have contributed to the degradation of extensive sloping landscapes, especially in the western and highland regions (Harper et al., 2007). These degraded sloping mountain ecosystems are characterized by pronounced physico-chemical and edaphic constraints, including soil erosion, rapid drainage, low water retention and reduced nutrient availability, which severely limit tree species establishment and long-term survival (Vågen et al., 2005).

Restoring vegetation cover in such environments is a priority not only for biodiversity conservation but also for erosion control and watershed protection. However, the successful establishment of woody species in these degraded sloping systems remains challenging. Seedlings are exposed to multiple environmental stresses from the earliest stages of development. In this context, improving plant performance both at the nursery stage and after field transplantation is a key challenge for restoration programs.

Among the biological approaches explored to enhance plant establishment, plant–microorganism interactions have received increasing attention, especially arbuscular mycorrhizal fungi (AMF), obligate symbiotic organisms associated with the roots of most terrestrial plant species across a wide range of ecosystems (Smith & Read, 2008), which play a central role. Through this symbiosis, AMF enhance plant nutrient acquisition, particularly phosphorus, improve water uptake, and increase plant tolerance to various abiotic and biotic stresses (Augé, 2001). Furthermore, AMF contribute to soil aggregation and stabilization, which may indirectly support plant establishment in erosion-prone environments (Rillig & Mummey, 2006; Mardhiah et al., 2016). Consequently, AMF are widely recognized as key drivers of plant performance and ecosystem functioning.

Beyond their general effects on plant growth, AMF have also been implicated in ecological processes such as plant community structuring and facilitation. Interactions involving mycorrhizal fungi can contribute to the selection of nurse plants, thereby promoting the establishment of other species in degraded environments (Baohanta et al., 2012; Baohanta et al., 2025). These properties make AMF promising candidates for use in ecological restoration strategies, particularly in nutrient-poor and physically constrained ecosystems.

However, AMF communities are not functionally uniform. Accumulating evidence suggests that their composition and structure are shaped by environmental conditions (Junkins at al., 2022; Lekberg et al., 2007). These variations may, in turn, affect the functional efficiency of

AMF in supporting plant development. In degraded ecosystems, native AMF communities may therefore represent locally adapted functional assemblages that could be mobilized to improve restoration outcomes. Despite this potential, the extent to which site-specific soil conditions influence AMF community structure, and how this in turn affects plant performance, remains insufficiently explored, particularly in tropical degraded sloping environments (Legesse et al., 2024).

Another important limitation concerns the transferability of AMF effects from controlled conditions to real field environments. While numerous studies have demonstrated that AMF inoculation can significantly enhance plant growth under nursery conditions, it remains unclear whether these benefits persist after transplantation, especially under the harsh environmental conditions typical of degraded sloping ecosystems (Dushimimana et al., 2022; Tejakusuma et al., 2023). Understanding this transition from nursery performance to field establishment is essential for evaluating the operational relevance of AMF-based approaches in restoration programs.

K. madagascariensis Jum. & H. Perrier, an endemic tree species of Madagascar, is widely used in anti-erosion and restoration programs due to its ecological value and adaptability. Despite its importance, little is known about its interaction with native AMF communities in degraded sloping environments, particularly in the western and highland regions of the island. Furthermore, the potential of native AMF inoculation to improve both early plant development and subsequent field establishment has not been sufficiently investigated for this species.

In this context, this study addresses the following question: Do site-specific soil conditions and native AMF communities shape nursery performance and field establishment of an endemic restoration tree?

We hypothesized that (i) site-specific soil chemical properties are associated with differences in the structure of native AMF communities, (ii) native AMF consortia derived from these communities enhance seedling growth under nursery conditions, and (iii) the positive effects observed under nursery conditions translate into improved plant establishment and survival after transplantation in degraded sloping sites, although their magnitude may vary depending on local environmental conditions.

The objective of this study was to investigate how site-specific soil conditions and native AMF communities influence the performance of *K. madagascariensis* from nursery to field conditions in degraded sloping ecosystems of Madagascar, with the aim of contributing to the development of site-adapted and ecologically relevant restoration strategies.

Materials and methods

Study sites and soil characterization

The study was conducted in two regions located in western Madagascar, namely Boeny (16.03278°S, 46.85680°E) and Betsiboka (17.23749°S, 46.92505°E). These regions are characterized by degraded sloping landscapes affected by erosion and low vegetation cover. For each region, the nursery was established near the corresponding planting site to maintain local environmental conditions.

Nursery substrates were collected from soils located in the vicinity of each study site to reflect local edaphic conditions and ensure ecological consistency between nursery production and field transplantation.

Field experiments were conducted in each region using eight plots (50 × 50 m) established on degraded sloping areas, hereafter referred to as Bo (Boeny) and Bt (Betsiboka). The sites consisted of relatively homogeneous herbaceous formations located on hills above 800 m

altitude, with slopes ranging from 15% to 40%. Soils in these areas originate from the weathering of sedimentary rocks and are influenced by erosion and landslide processes.

Soil sampling was carried out in both nursery substrates and field plots to characterize the environmental conditions at the two stages of plant development. This approach aimed to provide a baseline description of edaphic conditions and to support the interpretation of potential relationships between soil properties, native AMF communities and plant performance.

The following soil chemical properties were analyzed: pH (H_2O), total carbon (C), total nitrogen (N), organic matter content, C/N ratio, total phosphorus and available phosphorus. These parameters were determined for both nursery substrates and field planting sites.

Characterization of native AMF communities

To assess the structure of native arbuscular mycorrhizal fungal (AMF) communities associated with *Khaya madagascariensis*, rhizospheric soil samples were collected from naturally occurring individuals at the two study sites. For each site, five individual trees were selected based on their accessibility and spatial distribution to capture local variability. Individuals of comparable size and developmental stage were preferentially selected to reduce variability related to plant age. Sampling points were also distributed across the site to avoid spatial clustering of samples.

For each selected individual, rhizospheric soil was collected from the upper soil layer (0–20 cm) after carefully exposing the root system. Soil samples were taken near fine roots to ensure that the collected material corresponded to the rhizosphere. All samples were stored in plastic bags, transported to the laboratory under ambient conditions, and processed within a short time after collection.

Fine root samples were collected from the same individuals and processed according to the method of Phillips and Hayman (1970) to confirm the presence of mycorrhizal colonization through microscopic observation.

AMF spores were extracted from 100 g soil subsamples using the wet sieving method described by Gerdemann and Nicolson (1963). Each sample was suspended in 1 L of tap water, stirred vigorously and allowed to settle for 1 minute. Then, the soil suspension was poured onto four stacked sieves of 200, 100, 80 and 50 μm. This procedure was repeated five times to ensure that most spores contained in the soil sample passed through the sieves. The material retained on each sieve was transferred to 50 mL centrifuge tubes filled with distilled water and centrifuged at 3500 rpm for 10 min. The supernatant from each tube was collected in another 50 mL centrifuge tube, and a 60% sucrose solution was added before a second centrifugation at 3500 rpm for 1 min. The supernatants were filtered through a filter membrane. Spores on each filter were then counted under a stereomicroscope (40×) and grouped according to their morphological characteristics. They were classified and counted based on their size, color and shape.

Spore density was calculated as the number of spores per 100 g of dry soil. Morphological description of spores was conducted according to the standard color chart of glomalean fungi established by the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM). The color chart was used under the same illumination as for spore observation. For anatomical identification, a single intact AM spore was first mounted on a glass slide and stained with polyvinyl lactophenol (PVLG). Then, crushed spores were mounted between slide and coverslip with PVLG solution and PVLG containing Melzer's reagent and observed under a microscope.

Identification criteria followed Walker (1983), Schenck and Perez (1990), Morton (1992), Frank and Morton (1994), Muthukumar et al. (2009) and INVAM, considering spore

morphology, arrangement, size, shape, color, subtending hyphae forms, wall ornamentation, wall layer structures, staining reaction, and spore contents.

AMF inoculum preparation

Three AMF species per site were selected for the composite inoculum. The selection was based on the following criteria: (i) high relative abundance at the respective site, (ii) distinct correlation patterns with soil chemical properties, (iii) ecological representativeness, and (iv) high efficiency in infecting a mycotrophic host plant and promoting its growth. The latter criterion will be addressed in a separate publication.

The selected AMF isolates were individually propagated on leek (*Allium porrum*) under glasshouse conditions, using Terragreen (Oil Dri UK) as the propagation substrate. This substrate consists of attapulgite, a calcined clay with an average particle size of 5 mm, which is commonly used for AMF multiplication (Plenchette et al., 1996).

After 12 weeks of cultivation, the leek plants were uprooted and gently washed. The roots were then cut into 0.5 cm fragments containing approximately 250 vesicles per centimeter. Non-mycorrhizal leek roots prepared under the same conditions were used for the non-inoculated control treatment.

The mycorrhizal inoculum consisted of a mixture of spores (20–30 spores g^{-1}), colonized root fragments, external mycelium, and propagation substrate. For each site, a composite inoculum was prepared by mixing the three selected AMF types. This approach was adopted to mimic natural community conditions and to evaluate the collective functional effect of dominant native AMF assemblages on plant development.

Nursery experiment

Nursery experiments were conducted to evaluate the initial response of *K. madagascariensis* seedlings to AMF inoculation under controlled conditions. Pre-germinated seeds were planted in pots filled with nursery soil, and inoculation was performed by placing 1 g of AMF inoculum into the planting hole prior to sowing. Control treatments received non-mycorrhizal root fragments prepared under the same conditions.

After three months of growth, plant height and biomass parameters were measured. Fifty plants per treatment were harvested to determine shoot biomass, root biomass, and total biomass after oven-drying at 65°C for one week.

Root samples were collected to assess mycorrhizal colonization rates using microscopic observation following standard staining procedures (Phillips and Hayman, 1970). The percentage of colonized root length was determined for each plant.

Field experiment

Field experiments were conducted to assess whether the effects observed under nursery conditions translated into improved plant establishment under natural environmental constraints.

In each region, experimental plots were established on degraded sloping areas. A total of eight subplots (50 × 50 m) were set up per site, including four inoculated subplots and four non-inoculated control subplots. Seedlings previously grown in the nursery were transplanted into the field and assigned to subplots according to treatment. Each subplot contained 50 plants subjected to the same treatment conditions. Plants were planted at regular spacing within each subplot to ensure comparable growing conditions.

The distribution of subplots within each site followed a randomized design to minimize spatial bias. Subplots were spatially distributed across the site to reduce potential environmental heterogeneity. The subplot was considered as the experimental unit, with four replicates per

treatment per site. The Figure 1 summarizes the experimental design showing the randomized distribution of eight subplots per site.

Six months after transplantation, plant height was measured from the base of the stem to the tip of the apical shoot, and mortality rates were recorded for each subplot. Mortality rate was calculated as the percentage of dead plants relative to the total number of individuals initially planted in each subplot. These measurements were used to assess plant establishment and survival under field conditions.

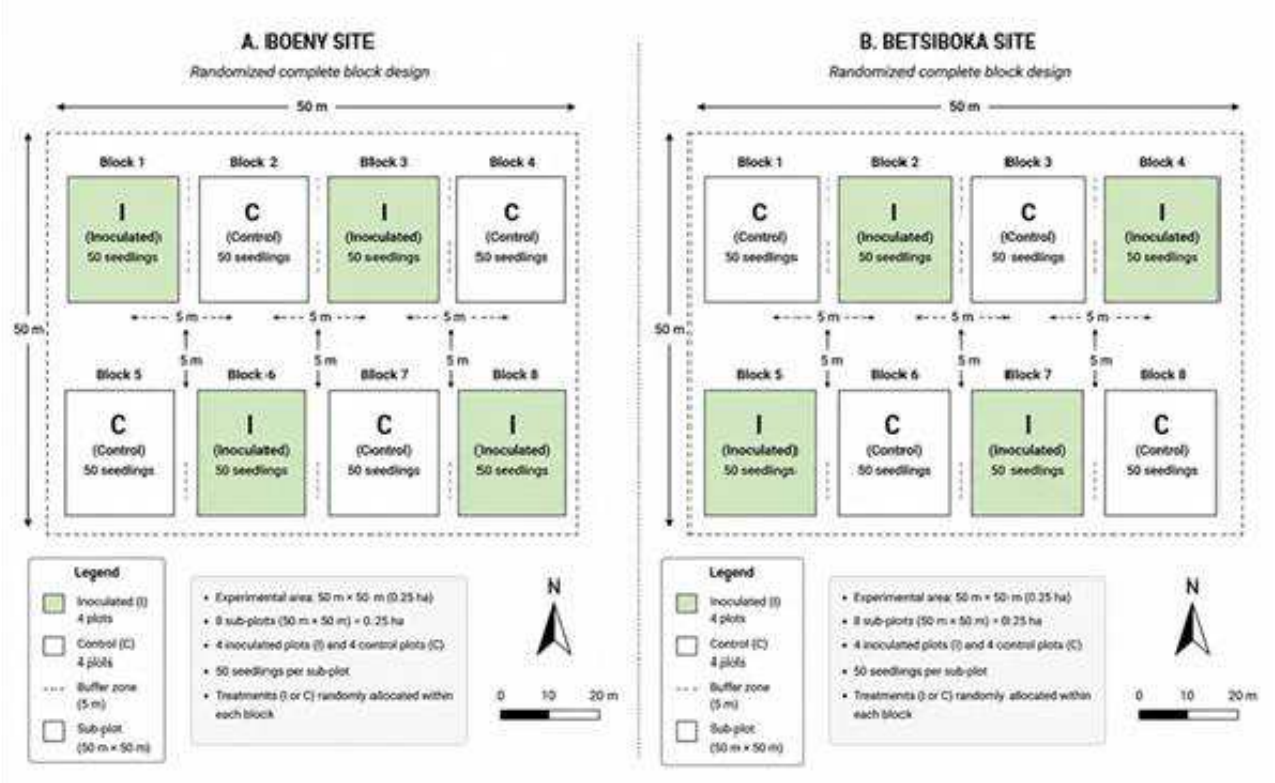


Figure 1. Experimental design showing the randomized distribution of eight subplots per site (Boeny and Betsiboka), including four inoculated (I) and four control (C) Blocs. Each bloc (50 × 50 m) contained 50 plants and was considered as the experimental unit.

Data analysis

Data analysis was designed to evaluate the effects of site-specific AMF inoculation on seedling performance from nursery to field conditions, and to examine how these responses varied between sites.

Soil chemical properties were analyzed using descriptive statistics to compare edaphic conditions between Boeny and Betsiboka, both at the nursery stage and under field conditions. Given the limited number of sites, differences were interpreted in terms of ecological contrasts rather than subjected to formal statistical testing.

For AMF communities, spore density (number of spores per 100 g of dry soil), relative abundance of each taxon, species richness (Shannon index, H'), and evenness were calculated for each site. Differences in community composition between sites were interpreted qualitatively and supported by correlation analyses with soil chemical properties using Spearman's rank correlation coefficients. Heatmaps were used to visualize relationships between AMF taxa and soil variables, and significance levels were determined at $p < 0.05$.

For nursery experiments, response variables included shoot biomass, root biomass, total biomass, and mycorrhizal colonization rate. Differences between inoculated and non-inoculated treatments were assessed using one-way analysis of variance (ANOVA) after verification of normality and homoscedasticity of residuals. When necessary, biomass data were log-transformed to meet model assumptions. Mycorrhizal colonization rates were arcsine square-root transformed prior to analysis. Differences were considered statistically significant at $p < 0.05$.

For field experiments, plant performance after transplantation was assessed using continuous response variables measured at the individual plant level. Differences between inoculated and control treatments within each plot were evaluated using Welch's *t*-tests, which do not assume

equal variances. In addition to statistical significance, the magnitude of inoculation effects was quantified by calculating the mean difference between treatments (Δ performance = inoculated − control) for each plot. Confidence intervals (95%) were computed to assess the robustness of treatment effects.

Although the subplot was defined as the experimental unit in the experimental design, individual-level analyses were used here to explore variability in plant responses and to provide a detailed representation of treatment effects within plots. This approach was complemented by effect size estimation to ensure a robust interpretation of inoculation impacts.

The consistency between nursery and field responses was assessed by comparing the direction and relative magnitude of inoculation effects across stages. All statistical analyses were performed using XLSTAT 2025 (Addinsoft, Paris, France) and R software (version 4.3.1). Significance thresholds were set at $p < 0.05$.

Results

Variability of chemical properties of the two study sites

Table 1 summarizes the results of the analyses of variance for the substrate (soils) used in the nursery across the two sites.

Table 1. Chemical characteristics of the nursery substrate from the two study sites (Boeny and Betsiboka).

	pH	Total_C	N	OM	Total_P	Available_P
Boeny Ns	5.10 b	1.36 b	0.17 a	1.81 b	15.03 a	4.92 b
Betsiboka NS	5.23 a	1.80 a	0.09 b	1.92 a	14.53 b	5.37 a

Different letters within the same column indicate significant differences at p < 0.05 (Kruskal-Wallis followed by Tukey HSD test).

The analysis of the physicochemical properties of the nursery substrate revealed significant differences between the two sites for all measured variables. For Boeny, the nursery substrate exhibited a significantly lower pH (5.10 b) compared to Betsiboka (5.23 a), as well as lower total carbon (1.36 b) and higher total nitrogen (0.17 a). In contrast, the Betsiboka nursery substrate showed significantly higher organic matter (1.92 a), lower total phosphorus (14.53 b), and higher available phosphorus (5.37 a). These chemical contrasts suggest differences in baseline fertility and nutrient availability between the two sites, which may subsequently influence both AMF functioning and seedling development in the nursery.

The following table (Table 2) summarizes the results of variance analyses for the field soil used at the two sites.

Table 2. Chemical properties of the field soil from the Boeny and Betsiboka sites.

	pH	**Total_C**	**N**	**OM**	**Total_P**	**Available_P**
Boeny FS	5.79 a	0.94 a	0.09 a	1.61 a	14.2 a	2.62 b
Betsiboka FS	5.51 b	0.83 b	0.08 b	1.44 b	11.5 b	3.54 a

Different letters within the same column indicate significant differences at $p < 0.05$ (Kruskal-Wallis followed by Tukey HSD test).

The statistical analysis of the field soil properties revealed significant differences between the two sites for all measured parameters. The Boeny field soil showed a significantly higher pH (5.79 a) compared to Betsiboka (5.51 b), along with higher total carbon (0.94 a), total nitrogen (0.09 a), organic matter (1.61 a), and total phosphorus (14.2 a). Conversely, the Betsiboka field soil exhibited a significantly higher available phosphorus content (3.54 a) despite having lower total phosphorus (11.5 b). These contrasting physicochemical profiles indicate that the two sites possess inherently different soil fertility characteristics, which may have important implications for nutrient management and plant performance under field conditions.

Table 3. Comparison of chemical properties between the nursery substrate (NS) and the field soil (FS) at the Boeny site.

	pH	Total_C	N	OM	Total_P	Available_P
Boeny Ns	5.10 b	1.36 a	0.17 a	1.81 a	15.03 a	4.92 a
Boeny FS	5.79 a	0.94 b	0.09 b	1.61 b	14.2 b	2.62 b

Different letters within the same column indicate significant differences at p < 0.05 (Kruskal-Wallis followed by Tukey HSD test).

At the Boeny site, the nursery substrate and the field soil showed significant differences for all measured parameters ($p < 0.05$) (Table 3), supporting the hypothesis that they are chemically distinct. On one hand, the nursery substrate showed significantly lower pH (5.10 b) but higher total carbon (1.36 a), total nitrogen (0.17 a), and organic matter (1.81 a) compared to the field soil. On the other hand, the field soil showed significantly lower total phosphorus (14.2 b) and lower available phosphorus (2.62 b), while the nursery medium contained higher total phosphorus (15.03 a) and significantly higher available phosphorus (4.92 a). These contrasting nutrient dynamics, particularly the inverse relationship between total and available phosphorus, suggest that plants grown in the nursery may experience different nutritional conditions than those encountered after transplanting to the field.

Concerning the Betsiboka site, Table 4 compares the chemical properties of the nursery substrate and the field soil.

Table 4. Comparison of physicochemical properties between the nursery growing medium (GM) and the field soil (FS) at the Betsiboka site

	pH	Total_C	N	OM	Total_P	Available_P
Betsiboka NS	5.23 b	1.80 a	0.09 a	1.92 a	14.53 a	5.37 a
Betsiboka FS	5.51 a	0.83 b	0.08 b	1.44 b	11.5 b	3.54 b

Different letters within the same column indicate significant differences at p < 0.05 (Kruskal-Wallis followed by Tukey HSD test).

We recorded that for the Betsiboka site, the nursery substrate and the field soil also displayed significant differences for all measured parameters ($p < 0.05$), further supporting the hypothesis that the two substrates are chemically distinct. The nursery substrate exhibited significantly

lower pH (5.23 b) but substantially higher total carbon (1.80 a), organic matter (1.92 a), total phosphorus (14.53 a), and available phosphorus (5.37 a) compared to the field soil. In contrast, the field soil showed higher pH (5.51 a) but lower values for all other variables, including total carbon (0.83 b), nitrogen (0.08 b), organic matter (1.44 b), total phosphorus (11.5 b), and available phosphorus (3.54 b). Unlike at Boeny, where the nursery medium had higher total phosphorus and higher available phosphorus, Betsiboka's nursery medium was also superior in both total and available phosphorus. These site-specific contrasts confirm the lack of chemical similarity between the nursery substrates and the field soils, a condition that may have significant repercussions for seedling acclimatization and post-transplant survival.

Dynamics of Arbuscular Mycorrhizal Fungi community within the two sites

The following tables (Table 5 et table 6) and the figure 2 present the dynamics of arbuscular mycorrhizal fungi (AMF) spore communities at the two study sites, including relative abundance (AR) and frequency (FR) of identified species, as well as spore density, species richness (H'), and evenness.

Table 5. Relative abundance (AR, %) and frequency (FR, %) of AMF species at the Boeny and Betsiboka sites.

	Betsiboka AR (%)	Boeny AR (%)	Betsiboka FR (%)	Boeny FR (%)
Glomus ambisporum	20.76	15.56	100%	100%
Gigaspora decipiens	4.67	12.04	100%	100%
Rhizophagus aggregatus	12.90	3.94	100%	100%
Acaulospora caspicula	41.15	63.53	100%	100%
Diversispora spurca	5.71	4.93	100%	100%
Scuttelospora dipurpurascens	14.80	0.00	100%	0%

Table 6. Spore density, Shannon diversity index (H'), and evenness of AMF communities at the Boeny and Betsiboka sites

Site	Spores density	H'	Evenness

Boeny	3 674.4 ± 536.8	1.107	0.688
Betsiboka	5 900.4 ± 600.8	1.545	0.862

328

329 We observed that there is significant difference between AMF community structure of the two
330 sites, consistent with the previously observed physicochemical contrasts between Boeny and
331 Betsiboka. Acaulospora caspicula dominated both sites in relative abundance, although it was
332 more prevalent at Boeny (63.53%) than at Betsiboka (41.15%).

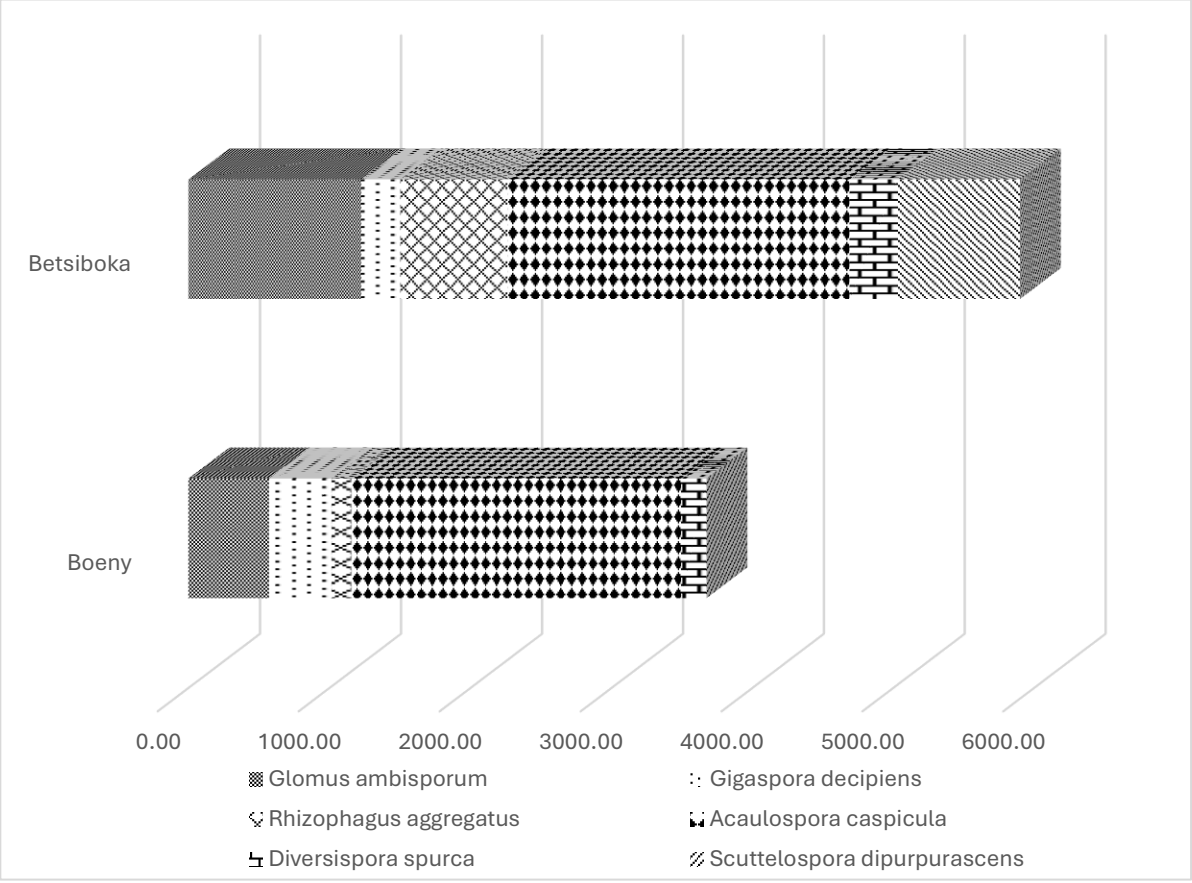


Fig. 2. The relative abundance of AM fungi genera within the two sites

333

334 However, Scuttelospora dipurpurascens was entirely absent from Boeny (0% AR and 0% FR)
335 but present at Betsiboka (14.80% AR, 100% FR), suggesting a site-specific exclusion or
336 ecological preference. All other species occurred at both sites with 100% frequency, though
337 their relative abundances varied. Regarding community metrics, Betsiboka exhibited

substantially higher spore density (5 900.4 ± 600.8 vs. 3 674.4 ± 536.8), greater species diversity (H' = 1.545 vs. 1.107), and higher evenness (0.862 vs. 0.688) compared to Boeny. These differences suggest that the Betsiboka site provides more favorable conditions for AMF proliferation and community evenness, possibly linked to its distinct soil chemical properties, particularly higher organic matter and available phosphorus, as reported earlier.

The following heatmap will further explore the correlations between these AMF parameters and the chemical properties of the soils.

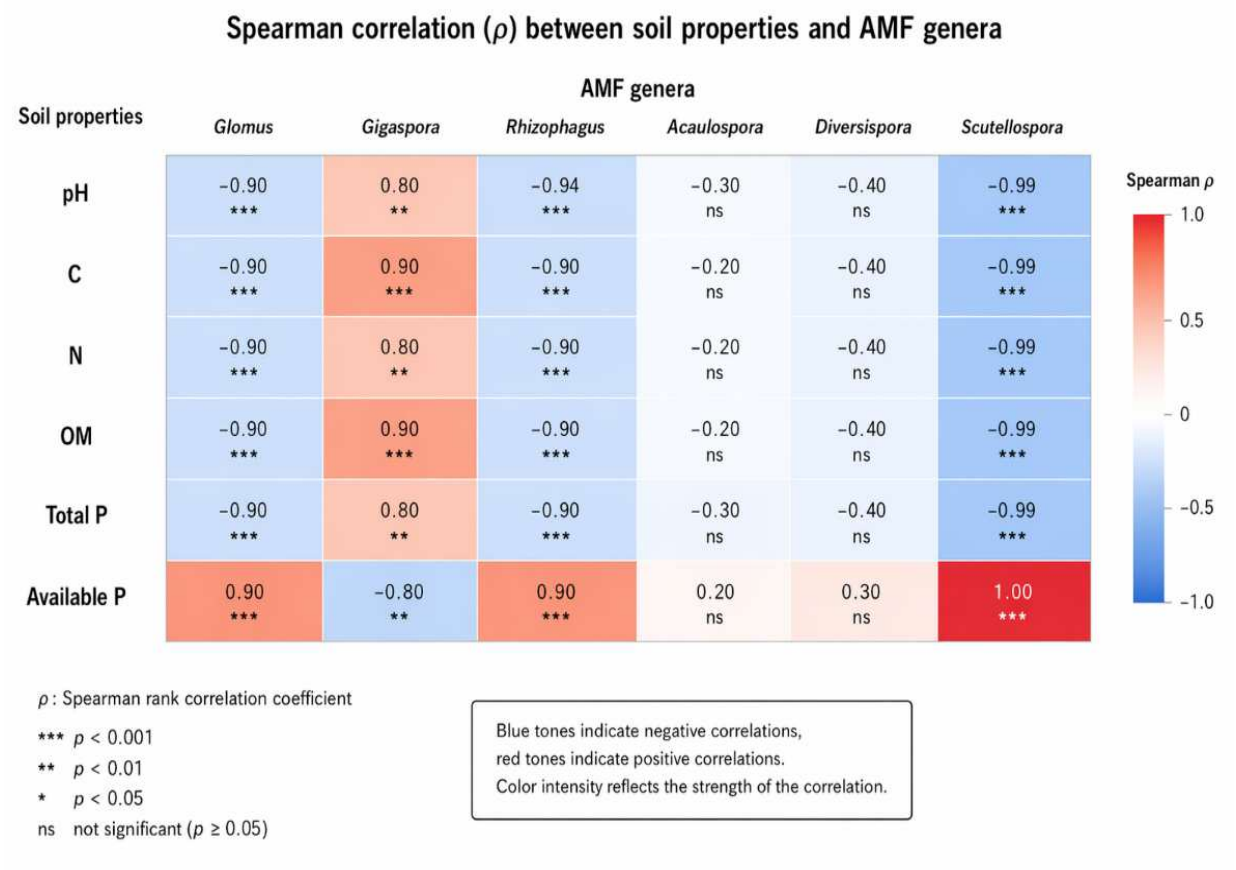


Figure 3. Heatmap of Spearman correlation p between soil chemical properties and AMF genera. Significance levels are indicated as follows: ns not significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Contrasting response patterns among AMF genera were observed in relation to soil chemical properties, as revealed by Spearman correlation analysis (Figure 3). A first group of species, *Glomus ambisporum, Rhizophagus aggregatus, Scutellospora dipurpurascens*, and to a lesser extent *Diversispora spurca*, exhibited strong negative correlations with pH, total carbon, nitrogen, organic matter, and total phosphorus (ρ ranging approximately from −0.78 to −0.93, with most correlations significant at $p < 0.01$ or $p < 0.001$). These results indicate that these taxa are preferentially associated with more acidic and nutrient-poor soil conditions. In contrast, these same species displayed positive correlations with available phosphorus, particularly *Rhizophagus aggregatus* and *Scutellospora dipurpurascens* ($\rho > 0.70$, $p < 0.01$), suggesting a strong responsiveness to readily available phosphorus despite their negative association with total phosphorus. This pattern highlights the importance of phosphorus bioavailability rather than total phosphorus content in structuring AMF community composition. *Gigaspora decipiens* exhibited an opposite trend, showing positive correlations with pH, total carbon, and organic matter, and negative correlations with available phosphorus, indicating a distinct ecological strategy potentially associated with more fertile soil conditions. *Acaulospora caspicula* showed weak and non-significant correlations with all measured soil parameters (ρ close to zero), confirming its ecological plasticity and supporting its classification as a generalist species, largely independent of the edaphic variations observed between the two sites.

Overall, these results suggest the existence of distinct ecological strategies among AMF genera, ranging from soil-sensitive taxa strongly structured by edaphic conditions to generalist taxa with broad ecological tolerance.

These findings directly informed the selection of AMF species for the development of composite inocula. For the Boeny site, the combination of *Acaulospora caspicula* (generalist and dominant), *Glomus ambisporum* (representative of soil-sensitive taxa), and *Gigaspora decipiens* (functionally complementary) was retained. For the Betsiboka site, *Acaulospora*

caspicula was again selected to ensure cross-site comparability, together with *Scutellospora dipurpurascens* (site-specific and strongly associated with available phosphorus) and *Rhizophagus aggregatus* (highly responsive to phosphorus availability).

This selection strategy was designed to integrate both functional diversity and site-specific ecological adaptations, thereby maximizing the relevance of the composite inoculum for subsequent nursery and field performance assessments. The results of the greenhouse efficiency tests of these selected AMF consortia will be presented in a separate study.

Inoculated plant development under nursery conditions

The following figure (Figure 4) presents the effect of AMF inoculation on plant biomass under nursery conditions at the Boeny site.

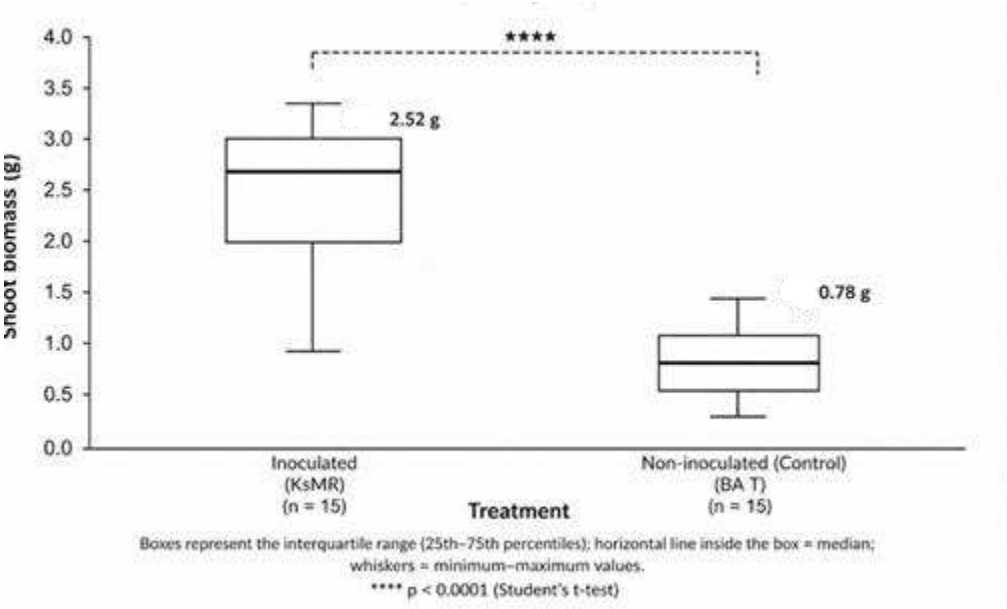


Figure 4. Effect of AMF inoculation on shoot and root biomass of plants grown in nursery conditions at the Boeny site.

Inoculation with the composite AMF inoculum significantly enhanced plant biomass production at the Boeny site under nursery conditions. For shoot biomass, inoculated plants showed a highly significant increase compared to non-inoculated controls ($p < 0.0001$).

Similarly, root biomass was also significantly improved by AMF inoculation ($p < 0.01$). The boxplot distributions indicate that inoculated plants consistently achieved higher median values for both shoot and root biomass, with a relatively compact interquartile range suggesting a uniform positive response to inoculation. In contrast, non-inoculated plants exhibited lower biomass production with wider variability. These results demonstrate that the selected AMF species combination, *Acaulospora caspicula, Glomus ambisporum*, and *Gigaspora decipiens*, effectively promotes plant growth in the Boeny nursery growing medium, supporting the hypothesis that native AMF communities can enhance seedling vigor under controlled conditions.

The following figure (Figure 5) presents the effect of AMF inoculation on shoot biomass under nursery conditions at the Betsiboka site.

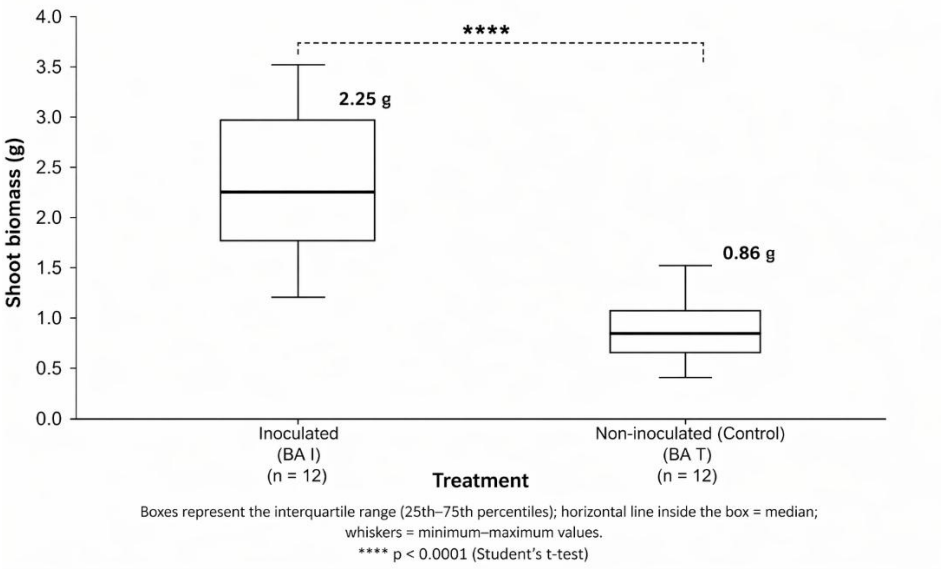


Figure 5. Effect of AMF inoculation on shoot biomass of plants grown in nursery conditions at the Betsiboka site.

At the Betsiboka site, AMF inoculation also resulted in a highly significant increase in shoot biomass compared to non-inoculated controls ($p < 0.0001$). The boxplot shows that inoculated

plants achieved distinctly higher median shoot biomass values, with a relatively tight interquartile range indicating a consistent positive response across replicates. In contrast, non-inoculated control plants exhibited markedly lower shoot biomass and greater variability. These results confirm that the selected AMF species combination for Betsiboka, *Acaulospora caspicula*, *Scutellospora dipurpurascens*, and *Rhizophagus aggregatus*, effectively promotes shoot growth under nursery conditions. Together with the results obtained at the Boeny site, these findings support the hypothesis that native, site-specific AMF consortia enhance seedling biomass production, although the magnitude of the response appears comparable between the two sites despite their contrasting soil chemical properties.

The following figure (Figure 6) presents the effect of AMF inoculation on root biomass and mycorrhization rate under nursery conditions at the Boeny site.

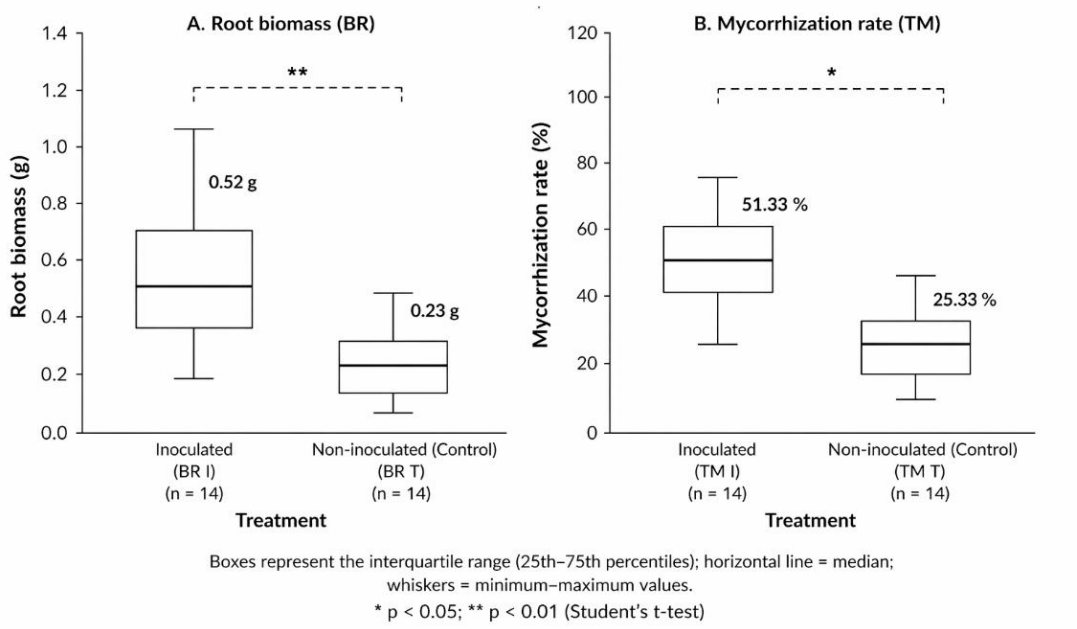


Figure 6. Effect of AMF inoculation on root biomass (A) and mycorrhization rate (B) of plants grown in nursery conditions at the Boeny site.

For the Boeny site, AMF inoculation significantly enhanced both root development and mycorrhization rate. Root biomass was significantly higher in inoculated plants compared to

non-inoculated controls ($p < 0.05$). More strikingly, the mycorrhization rate showed a highly significant increase in inoculated plants ($p < 0.01$), with inoculated plants reaching a median value approximately double that of the non-inoculated controls (0.52 vs 0.23, based on the figure). Non-inoculated plants exhibited low mycorrhization rates, indicating minimal background colonization from native AMF present in the nursery growing medium. These results confirm that the selected AMF consortium effectively colonizes plant roots at the Boeny site, and this successful root colonization is associated with improved root biomass production. The simultaneous increase in both parameters suggests a functional link between mycorrhization and root development, although formal correlation analysis would be required to establish this relationship.

For betsiboka, the figure 7 present the effect of AMF inoculation on root biomass and mycorrhization rate under nursery conditions at the Betsiboka site.

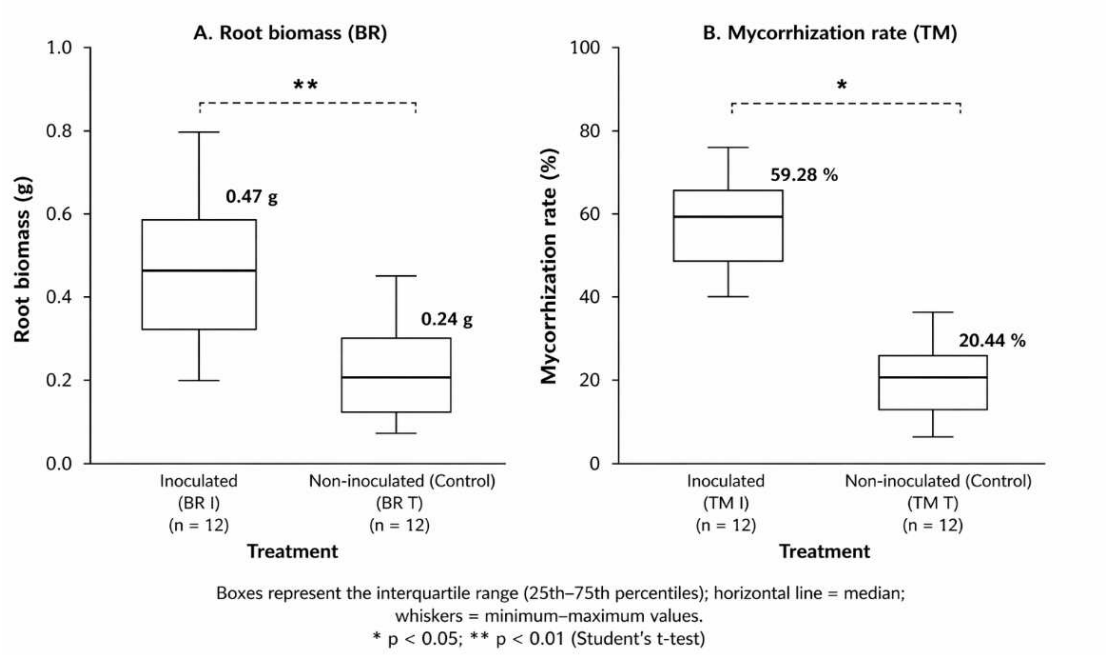


Figure 7. Effect of AMF inoculation on root biomass (A) and mycorrhization rate (B) of plants grown in nursery conditions at the Betsiboka site.

At the Betsiboka site, AMF inoculation significantly enhanced both root biomass and mycorrhization rate. Root biomass was significantly higher in inoculated plants compared to non-inoculated controls ($p < 0.05$), with inoculated plants reaching a median value of 0.47 g versus 0.24 g in controls. Similarly, the mycorrhization rate showed a highly significant increase in inoculated plants ($p < 0.01$), reaching approximately 59% compared to only 20% in non-inoculated controls. Non-inoculated plants exhibited a low but detectable mycorrhization rate (20.44%), suggesting the presence of background native AMF populations in the Betsiboka nursery growing medium, although at levels insufficient to promote root growth comparable to the inoculated treatment. These results confirm that the selected AMF consortium for Betsiboka, *Acaulospora caspicula*, *Scutellospora dipurpurascens*, and *Rhizophagus aggregatus* — effectively colonizes plant roots and significantly improves root biomass production. The simultaneous increase in both parameters suggests a functional link between mycorrhization and root development at Betsiboka, consistent with the pattern observed at Boeny.

Inoculation effect in planting site conditions

Survival rate

The impact of AMF mixture inoculum on the plant survival of *K.madagascariensis* in field plots for Boeny is presented by the figure 8.

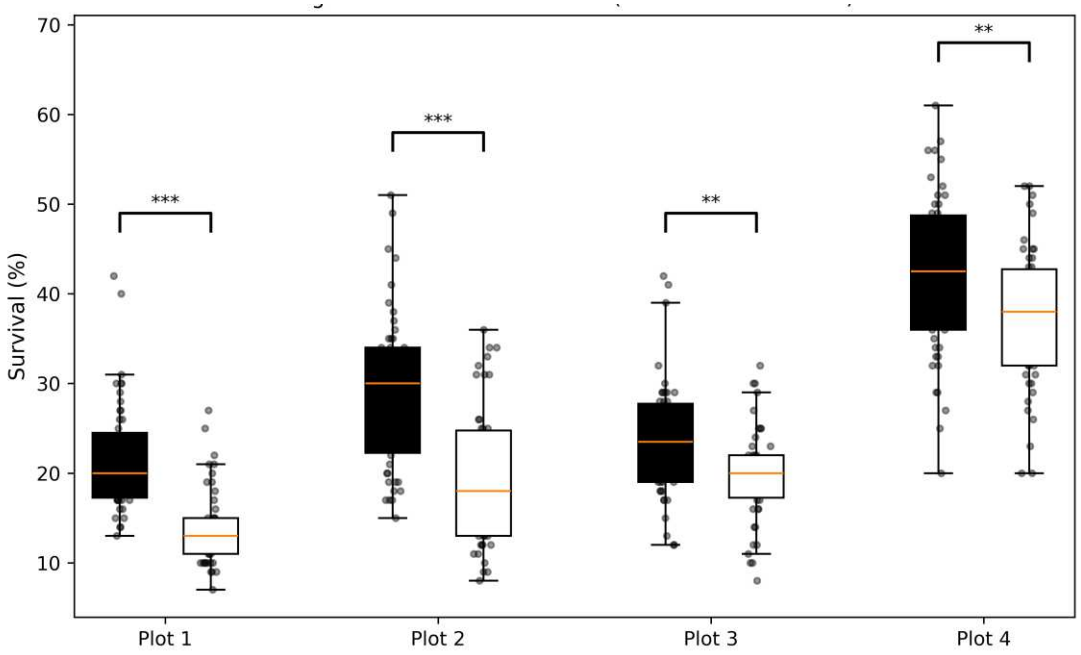


Figure 8. Distribution of post-transplant performance of *K. madagascariensis* seedlings according to inoculation treatment across experimental plots

Individual post-transplant performance values of seedlings measured under field conditions at each site. Data are presented for each plot (Plot 1–4) and treatment [Inoculated (Left) vs Control (Right); $n = 50$ per treatment per plot]. Boxplots represent the median (central line), interquartile range (IQR), and whiskers extending to 1.5 × IQR. Points correspond to individual seedlings.

Differences between treatments within each plot were assessed using Welch's *t*-tests. Significance levels are indicated as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Inoculation significantly enhanced the post-transplant performance of *K. madagascariensis* seedlings across all experimental plots at the Boeny site (Figure 8). In each plot, inoculated seedlings exhibited higher performance values than non-inoculated controls, with a clear upward shift in the distribution of individual responses.

These differences were statistically significant based on Welch's t-tests (Plot 1 and Plot 2: $p < 0.001$; Plot 3 and Plot 4: $p < 0.01$). The magnitude of the inoculation effect varied among plots, with the strongest responses observed in Plot 2 and Plot 1. In contrast, more moderate effects were detected in Plot 3 and Plot 4, where control seedlings already showed relatively higher baseline performance.

The effect size, expressed as the difference between inoculated and control seedlings (Δ performance), was consistently positive across all plots (Figure 9), indicating a robust beneficial effect of inoculation under field conditions. This pattern suggests that inoculation enhances seedling establishment, although its magnitude is modulated by local environmental conditions.

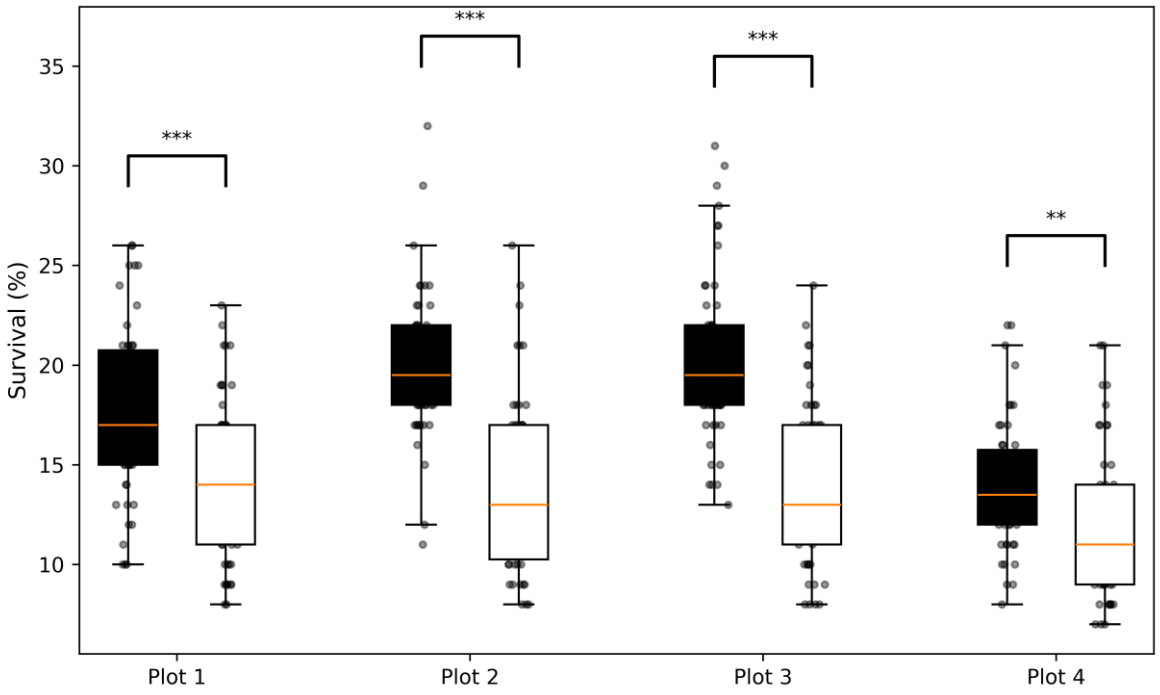


Figure 9. Effect of inoculation on seedling post-transplant performance [Δ performance = Inoculated (Left) – Control (Right)] across plots

Effect of inoculation expressed as the mean difference in performance between inoculated and control seedlings for each plot. Points represent mean differences (Δ performance), and error bars indicate 95% confidence intervals.

The dashed horizontal line represents no treatment effect (Δ = 0). Positive values indicate a beneficial effect of inoculation.

Statistical significance within each plot was assessed using Welch's *t*-tests and is indicated by asterisks: *** $p < 0.001$, ** $p < 0.01$.

At the Betsiboka site, inoculation also significantly improved the post-transplant performance of *K. madagascariensis* seedlings across all experimental plots (Figure 9). Inoculated seedlings consistently showed higher performance values than controls, with statistically significant differences in all plots (Plot 1–3: $p < 0.001$; Plot 4: $p < 0.01$).

However, the magnitude of the inoculation effect was generally lower than that observed at the Boeny site. Effect sizes ranged from moderate to low (approximately +2% to +6%), and the separation between treatments was less pronounced. Despite this, the effect remained consistently positive across all plots (Figure 9), confirming the beneficial role of inoculation under field conditions.

These results indicate that while inoculation improves seedling performance in both sites, its impact is context-dependent and influenced by site-specific environmental conditions.

Overall, inoculation effects were stronger at the Boeny site than at Betsiboka. This difference suggests that the effectiveness of AMF inoculation is modulated by environmental context, potentially reflecting variations in soil properties, native microbial communities, and resource availability between sites.

Discussion

The marked differences in soil chemical properties observed between Boeny and Betsiboka confirm that the two sites represent distinct edaphic environments, likely exerting strong selective pressures on belowground microbial communities. Variations in pH, organic matter, carbon, nitrogen and phosphorus availability are well-known drivers of AMF community composition and structure (Lekberg et al., 2007; Davison et al., 2015; Alguacil et al., 2016). In the present study, these edaphic contrasts were associated with clear differences in AMF community characteristics, including spore density, diversity and relative abundance of taxa.

Betsiboka exhibited higher spore density, diversity and evenness compared to Boeny, suggesting more favorable conditions for AMF proliferation and coexistence. In contrast, Boeny showed a more uneven community structure dominated by *Acaulospora caspicula*. This pattern supports the hypothesis that AMF communities are shaped by local soil conditions and may represent site-adapted functional assemblages. Such environmental filtering has been widely reported in tropical and degraded ecosystems, where soil constraints strongly influence microbial community assembly and functioning (van der Heijden et al., 2015; Powell & Rillig, 2018).

Beyond compositional differences, the observed variation in AMF taxa between sites highlights the existence of distinct ecological strategies among fungal genera. The dominance of *Acaulospora caspicula* at both sites suggests a generalist behavior with high ecological plasticity, as already reported for several *Acaulospora* species capable of persisting across a wide range of environmental conditions (Smith & Read, 2008).

In contrast, the presence of *Scutellospora dipurpurascens* exclusively at Betsiboka and the differential abundance of *Rhizophagus aggregatus* and *Gigaspora decipiens* indicate more specialized ecological niches and potential functional differentiation among taxa. These

differences are consistent with previous studies showing that AMF species vary in their efficiency of nutrient acquisition, hyphal development, and response to soil fertility gradients (Johnson et al., 1997; Hoeksema et al., 2010).

The combination of generalist and site-sensitive taxa in the selected inocula therefore represents a functionally relevant strategy, allowing both stability and adaptability of plant–microbe interactions. Such functional complementarity is increasingly recognized as a key factor for improving plant performance in restoration contexts (van der Heijden et al., 2015).

The strong positive effects of AMF inoculation on shoot biomass, root biomass and mycorrhizal colonization under nursery conditions confirm the functional efficiency of the selected native AMF consortia. These results support the second hypothesis and demonstrate that native AMF communities can significantly enhance early seedling development of *K. madagascariensis*.

The increase in root biomass and mycorrhization rate suggests improved belowground resource acquisition capacity, which is critical for plant establishment. AMF are known to enhance phosphorus uptake, improve water relations, and increase tolerance to abiotic stress (Augé, 2001; Smith & Read, 2008). The relatively uniform response observed across replicates further indicates that the inoculation effect is robust under controlled conditions.

These findings are consistent with numerous studies showing that pre-inoculation with AMF can significantly improve seedling vigor in nursery systems, thereby increasing their potential for successful transplantation (Koziol & Bever, 2016; Koziol et al., 2018).

One of the key outcomes of this study is the demonstration that the positive effects of AMF inoculation observed under nursery conditions are at least partially maintained after transplantation to field conditions. In both Boeny and Betsiboka, inoculated seedlings exhibited higher post-transplant performance than controls, indicating that AMF-mediated benefits can persist under natural environmental constraints.

This result is particularly important in the context of ecological restoration, as many studies report a decline or loss of inoculation effects after transplantation due to environmental stress, competition, and microbial interactions in the soil (Dushimimana et al., 2022; Tejakusuma et al., 2023). The persistence of a positive effect in this study suggests that the use of native AMF consortia may improve the ecological relevance and effectiveness of inoculation strategies.

However, the variability in effect magnitude between nursery and field conditions indicates that this transfer is not complete and may be influenced by environmental filtering processes occurring after transplantation.

Although inoculation had a consistently positive effect at both sites, its magnitude differed markedly between Boeny and Betsiboka, with stronger effects observed at Boeny. This pattern highlights the context dependency of plant–AMF interactions and supports the third hypothesis.

Such variability is well documented in the literature, where the outcome of mycorrhizal symbiosis depends on multiple factors, including soil fertility, phosphorus availability, environmental stress and compatibility between host plants and fungal partners (Johnson et al., 1997; Hoeksema et al., 2010). In nutrient-poor or stressful environments, AMF tend to provide greater benefits to host plants, whereas in more favorable conditions their relative contribution may decrease.

In this study, the weaker response observed at Betsiboka may be explained by higher baseline soil fertility, higher AMF diversity and potentially greater background colonization, which could reduce the relative advantage of inoculation. Conversely, the stronger response at Boeny suggests that AMF play a more critical role in supporting plant performance under more limiting conditions.

The results of this study provide strong evidence that integrating native AMF inoculation into restoration practices can enhance the performance of *K. madagascariensis* seedlings from

nursery to field conditions. By demonstrating both the effectiveness and the context dependency of AMF inoculation, this work highlights the importance of considering site-specific ecological conditions in restoration design.

Rather than applying uniform restoration strategies, the use of locally adapted AMF consortia appears to be a promising approach to improve plant establishment in degraded environments. This is particularly relevant in sloping ecosystems, where soil erosion, nutrient limitation and water stress severely constrain plant survival and growth.

More broadly, this study supports an integrated restoration framework that combines plant selection, soil characterization and microbial management. Such an approach aligns with emerging paradigms in restoration ecology emphasizing the role of plant–soil–microbe interactions in determining ecosystem recovery trajectories (van der Heijden et al., 2015; Powell & Rillig, 2018).

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