

Impact of *Psidium cattleianum* Invasion on Soil Microbial Functioning and on *Uapaca ferruginea* (Baill.) Regeneration at Forest Edge in the Eastern Part of Madagascar

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Abstract Perturbations caused by alien species on native plant regeneration and on soil microbial dynamics are major problem to biodiversity conservation. In this study, our main objective was to evaluate the impact of the propagation of an exotic plant species, *Psidium cattleianum*, i) on soil Arbuscular Mycorrhizal (AM) fungus dynamics, and ii) on the regeneration of *Uapaca ferruginea*, a native species in the Eastern forest of Madagascar. Soil samples were collected within (i) a homogenous formation of *P. cattleianum*, (ii) a natural forest devoid of *P. cattleianum* dominated mainly by *U. ferruginea*, and (iii) a degraded soil outside the forest and devoid of *P. cattleianum*. Soil enzymatic activities, soil microbial activity, the Most Probable Number (MPN) of mycorrhizal propagules in soil and the total number of Arbuscular Mycorrhiza spores in soil were assessed. Seedling development of *U. ferruginea* planted on these three soil samples were assessed after 5 months culturing under greenhouse conditions. The total numbers of AM Fungal spores were significantly high on colonized soil by *P. cattleianum*. Global microbial activity was significantly higher on natural forest soil than those recorded on the other soil samples. Seedling development of *U. ferruginea* decreased from soil forest to invaded soil by *P. cattleianum* and degraded soil.

Keywords Biological invasion, *Psidium cattleianum*, Native essence, Regeneration, *Uapaca ferruginea*, Madagascar

1. Introduction

The fragility of island ecosystems in respect to the spread of alien exotic plants currently deserves careful consideration. Indeed, after the destruction of ecological habitats, the introduction of alien species is now regarded as the second direct reason of imbalance in the plant structure in the world [1]. Thus, the intensification of national and international trade of plants because of their socio-economical, ornamental or food value, has promoted the introduction of alien plant species in many countries in the world [1, 2].

Propagation of these alien species disturbs some soil parameters affecting the development of native species such as mycorrhizal fungi functioning and structure, soil

enzymatic activities and disturbing the conservation of biodiversity [3]. Different biological mechanisms are responsible of these disturbances: (i) modification of physico-chemical properties and microbial structure and functioning of soil through rhizodeposition or litter effect and (ii) competition with native species to acquire nutrients or light [4-6]. Indeed, some alien species caused more or less important changes at ground level, creating unfavorable condition to the establishment of native species [7, 8]. Thus, these species represent a serious threat to biodiversity as they can completely replace native species by invading the surroundings where they have been introduced [9].

In the eastern part of Madagascar, a rapid establishment of *Psidium cattleianum* on degraded natural forests was observed during last decade. At the same time, surveys showed that the number of native plant species decreased significantly around the invaded areas by *Psidium cattleianum*, (Ramanankierana, unpublished data). In this coastal forest, *U. ferruginea* constitutes one of the valuable woody species.

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The main objective of this study was to assess the impact of *P. cattleianum* propagation on mycorrhizal fungi community associated with *U. ferruginea* and on the seedling development of this tree species in the eastern forest ecosystem of Madagascar.

2. Materials and Methods

2.1. Study Site Description

The study area is located in the forest ecosystem of Ianjomara-Vatomandry (S 19°07', E 48°54', 113 m), situated about 200 km east of Antananarivo, Madagascar. This forest represents one of the remaining forest fragments in this part of the island. Plots of 10 X 10 m have been materialized in this forest formation to obtain three microhabitats on the basis of plant composition: (i) natural forest dominated by *U. ferruginea* and devoid of *P. cattleianum*; (ii) forest edge with propagation of *P. cattleianum* (iii) degraded soil located outside the forest and devoid of both *U. ferruginea* and *P. cattleianum* and where vegetation consists mainly of herbaceous species. Three plots were established for each soil type. Soil samples from each plot were used to assess structure of mycorrhizal fungi communities, global microbial activities and soil enzymatic activities. They were prepared in the greenhouse for the plantation of *U. ferruginea*.

2.2. Assessment of AM Fungus Community Structure

Density and morphological diversity of arbuscular mycorrhizal fungi spores were determined using the method described by Sieverding *et al.* [10]. AM fungal spores were extracted from soil samples by wet sieving and decanting, followed by sucrose centrifugation [11]. After centrifugation, the supernatant was poured through a 50-mm sieve and rinsed with tap water. Spores were counted under a stereomicroscope and grouped according to morphological characteristics. The relative abundance of each fungal type was calculated per 100g of dry soil. Spore size and color were assessed in water under a stereomicroscope (Olympus SZ H10 research stereomicroscope) whereas spore wall structures and other attributes were observed on permanent slides prepared according to Azcon-Aguilar *et al.* [12] under a microscope connected to a computer with digital image analysis software. For enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil, the MPN was determined using the method described by Porter *et al.* in [13].

2.3. Measurement of pH and Soil Microbial Functionalities

For each type of soil, pH of a water soil suspension was determined. Total microbial activity in soil samples was measured using the fluorescein diacetate (3', 6', -diacetylfluorescein [FDA]) hydrolysis assay according to the method of Alef *et al.* in [14] in which fluorescein

liberated was assayed colorimetrically at 490 nm, after 1 h of soil incubation. Soil phosphatase activity was measured by absorbance reading at 400 nm following the method of [15] with p-Nitrophenyl Phosphate (pNPP) as an artificial electron acceptor to form para nitrophenol (pN) under acid and alkaline condition. Result was expressed as the released para nitrophenol per g of soil per hour.

2.4. Seedling Development of *U. ferruginea*

Seeds of *U. ferruginea* were surface sterilized with 70% of ethanol for 5 min. They were pre-germinated for 5 days in sterilized sands humidified with distilled water at 25°C in the dark. For each soil origin, plastic pots (4 cm diameter, 20 cm height) were filled with 250 g of soil and one pre-germinated seed of *U. ferruginea* was planted per pot. 50 replicates per treatment were established and pots were arranged in a randomized complete block design in the greenhouse and seedlings grown under natural light (daylight of approximately 12 h, average daily temperature of 25°C). They were watered regularly with tap water without fertilizer.

After 5 months of cultivation, measurement of the plants heights was made. Then the plants were harvested and the oven-dried weight (1 week at 65°C) of shoot was measured. Their entire root systems were washed under tap water. On each plant, the extent of AM colonization was assessed (Phillips & Hayman, 1970) and the total dry weight of root per plant (1 week at 65°C) determined.

2.5. Statistical Analysis

The statistical analysis of the qualitative and quantitative variables was either of mono-varied type, or of multivariate type using STATISTICA software. The Student test was used for the averages and the variances comparison and the Newman-Keuls test for the comparison between the rates and a significant value if $p < 0.05$.

3. Results and Discussion

3.1. Sample Soils Characteristics

The soil pH recorded in the invaded area is slightly higher than in the uninvaded soil but the difference is not really significant. Similarly, no relevant difference had been noticed in the global microbial activity. However, soil phosphatase activity was importantly higher on forest soil with *U. ferruginea* compared to those measured on the two other soils under both acid and alkaline conditions (Table 1). The high rate of acid phosphatase compared to that of alkaline phosphatase was due to the chemical characteristic of the soil (pH~5). Enzymatic activities are also disturbed by *P. cattleianum*. Thus, the rate of acid phosphatase measured on the invaded soil was even lower than that observed on degraded area.

The density of AM spores decreased from degraded area, *P. cattleianum* soil, to forest soil with *U. ferruginea* (Table

1). Sporulation can be a sign of stressed environments at certain point. Therefore high total number of endomycorrhizal spore in the soil can indicate that most of the endomycorrhizal fungi are in sleeping mode on invaded or degraded area. The evaluation of (MPN) for each type of soil confirms that constation. The MPN is negatively correlated to the total number of spore. The observed rate of MPN per 100g of soil on invaded soil was six times lower than that determined on the uninvaded forest soil. The installation of *P. cattleianum* on soil induces then the inhibition of the spore germination. Our result showed the negative effect of *Psidium cattleianum* propagation on soil microbial activities and endomycorrhizal characteristics.

Table 1. Chemical characteristic (pH), enzymatic activities and AM fungi community of the three soil types

	Degraded area	Forest soil under <i>P. cattleianum</i>	Forest soil under <i>U. ferruginea</i>
pH (H ₂ O)	5,43a*	5,51a	5,38a
Global microbial activity (µg of hydrolyzed fluorescein diacetate h ⁻¹ g ⁻¹ of soil)	9,915a	10,442a	12,965a
Acid phosphatase (µg of p-nitrophenol h ⁻¹ g ⁻¹ of soil)	129,682b	118,333a	253,968c
Alkaline phosphatase (µg of p-nitrophenol h ⁻¹ g ⁻¹ of soil)	23,968a	22,381a	62,539b
Total number of AM spores (per 100g of soil)	956b	844b	596a
MPN of propagules (per 100g of soil)	8,974a	25,7b	153,81c

(*) Data in the same line followed by the same letter are not significantly different according to the test of Newman-Keuls ($p < 0, 05$).

3.2. Plant Growth and AM Colonization

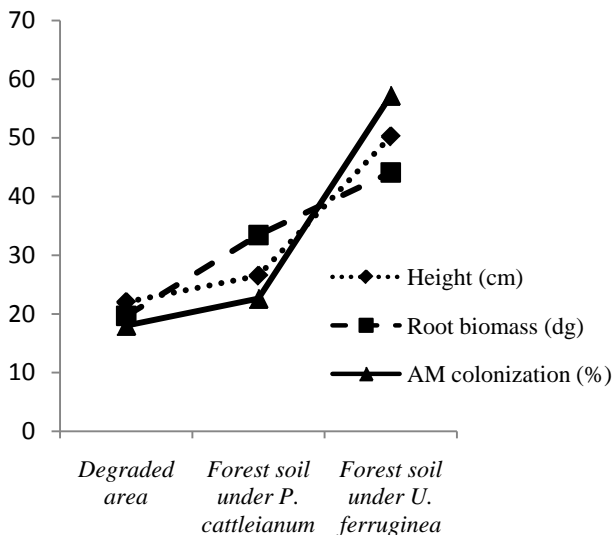


Figure 1. Development and AM colonization of *U. ferruginea* seedlings on the soil types after 5 months of cultivation

The growth response and AM colonization of *U. ferruginea* seedlings were significantly stimulated on forest soil without *P. cattleianum* than those observed on the other area as shown in Fig. 1. These results were shown according to the analysis of the height, the root biomass and AM colonization where we noticed a high value of each of these parameters on that type of soil. However, the result showed that the development of *U. ferruginea* seedlings on soil forest colonized by *P. cattleianum* was similar to that observed on degraded soil. Although the two samples soils were taken from the same forest, the growth of *U. ferruginea* on soil invaded had been reduced significantly.

In addition, the previously installation of *P. cattleianum* in the soil induced the reduction of AM colonization of *U. ferruginea*. The negative effect of invaded soil on AM colonization of the seedling was similar to that observed when it was grown in degraded area. These results indicate that the growth of *U. ferruginea* seedlings decrease with AM colonization. Oppositely, the increase of AM colonization accompanies the good development of the seedlings which confirm the role of AM fungi on seedling development as described by Barea et al. in [16].

4. Conclusions

This study provides evidence that the propagation of *P. cattleianum* can influence both soil microbial properties and native plant performance. The high competitive ability of this plant constitutes a real threat for biodiversity in the ecosystem. In the present study, the growth of *U. ferruginea* seedlings may be promoted by improving the AM colonization fungi.

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REFERENCES

- [1] Wells, M.J., Poynton, R.J., Balsinhas, A.A., Musil, K.J., Joffe, H., van Hoepen, E., Abbott, S.K., (1986): The history of introduction on invasive alien plants to southern Africa. In: Macdonald, I.A.W., Kruger, F.J., Ferrar, A.A. (Editor). The ecology and management of biological invasions in Southern Africa. Oxford University Press, Cape Town, South Africa, pp: 21-35.
- [2] Jauzen, Ph., (1998): Bilan des espèces naturalisées en France méditerranéenne. In: *Proceedings 6ème Symposium Méditerranéen EWRS, Montpellier, France*, 18-25.
- [3] Pyšek, P., Richardson, D.M., Rejmanek, M., Webster, G.L., Williamson, M and Kirschner, J., (2004): Alien plants in

- Checklists and floras. Towards better communication between taxonomists and ecologists. *Taxon*, 53: 131-143.
- [4] Vitousek, P.M., D'Antonio CM, Loope L.L., Rejmanek M., Westbrooks, R., (1997): Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, 21: 1- 16.
- [5] Rejmanek, M., (2000): Invasive plants: approaches and predictions. *Austral Ecology*, 25:497-5.
- [6] Sanon, A., Bguiristain, T., Cébron, A., Berthelin, J., Ndoye, I., Leyval, C., Sylla, S., Duponnois, R., (2009): Changes in soil diversity and global activities following invasions of the exotic invasive plant, *Amaranthus viridis* decrease the growth of native sahelian *Acacia* species. *FEMS Microbiol Ecol* 70:118–131.
- [7] Remigi, P., Faye, A., Kane, A., Deruaz, M., Thioulouse E, J., Cissoko, M., Prin, Y., Galiana, A., Dreyfus, B. & Duponnois, R., (2008): The exotic legume tree species *Acacia holosericea* alters microbial soil functionalities and the structure of the arbuscular mycorrhizal community. *Applied and Environmental Microbiology*, 74 (5): 1485-1493.
- [8] Kisa, M., Sanon, A., Thioulouse, J., Assigbetse, K., Sylla, S., Spichiger, R., Dieng, L., Berthelin, J., Prin, Y., Galiana, A., Lepage, M. & Duponnois, R., (2007): Arbuscular mycorrhizal symbiosis can counterbalance the negative influence of the exotic tree species *Eucalyptus camaldulensis* on the structure and functioning of soil microbial communities in a sahelian soil. *FEMS Microbiology Ecology*, 62 (1): 32-44.
- [9] Simberloff, D., (2003): Confronting introduced species: a form of xenophobia? *Biological Invasions*, 5: 179-192.
- [10] Sieverding, E., (1991): Vesicular-Arbuscular Mycorrhiza Management in Tropical *Agrosystems*. *G.T.Z., Eschborn, Germany*, 371 pp.
- [11] Gerderman, J.W. & Nicolson T.H., (1963): Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans Br Mycol Soc* 46: 235.
- [12] Azcon-Aguilar C., Palenzuela J.J., Roldan A., Bautista S., Vallejo R & Barea J.M., (2003): Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrub-lands. *Appl Soil Ecol* 14: 165-175.
- [13] Porter, W., (1979): The « Most Probable Number » method for enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. *Australian Journal of Soil Research* 17: .515-519.
- [14] Alef, K., (1998): Estimation of the hydrolysis of fluorescein diacetate. In: Alef K. and Nannipieri P. (Editor), *Methods in applied soil microbiology and biochemistry*. Academic Press, London, pp. 232 – 233.
- [15] Tabatabai M.A., (1982): Soil enzymes. In *Methods of Soil Analysis*. Part 2. (A. L. Page, R. H. Miller and D. R. Keeney, eds.). *Agronomy* 9: 903-947.
- [16] J.M Barea, J. Palenzuela, P. Cornejo, I Sanchez Castro, C Navarro-Fernandez, A Lopez Garcia, B. Estrada, R. Azcón, N. Ferrol, R. Azcón-Agvilar (2011): Ecological and functional roles of mycorrhizas in semi-arid ecosystems. *J. of Arid Environments*, 75 (12): 1292-1301.