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Propagation of *Grevillea banksii* Affects the Dynamic of Mycorrhizal Fungi Communities Associated with Native Tree Species of Madagascar

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Abstract: Propagation of exotic plant species is found in many regions of Madagascar Island. This work aims to describe the impacts of the propagation of *Grevillea banksii* on soil microbial activities and on the regeneration of two native tree species (*Intsia bijuga* and *Dalbergia trichocarpa*) in the eastern part of Madagascar. The study was conducted within Ianjomara forest where some types of the vegetation are observed such as an area characterized by grassland (P1), by homogeneous population of *G. banksii* (P2) and by a natural forest composed mainly of *I. bijuga* or *D. trichocarpa* (P3 and P4). Structure of mycorrhizal fungi communities and associated microorganisms were described on soils from each study plot. The development of *I. bijuga* and *D. trichocarpa*, was evaluated 4 months after planting on P1, P2, P3, P4 soils formerly colonized by *G. banksii*. According to the nutrients availability on each soil type, the development of *G. banksii* was accompanied or not by a high formation of cluster roots. The authors' results also show that soil occupied by *G. banksii* decreased the global microbial and phosphatase activities of soil especially on soil within a high density of cluster roots. Moderately mycotrophic, *G. banksii* disturbs the structure and the dynamics of symbiotic microflora such as endomycorrhizal fungi (MA) and rhizobia associated with the two native tree species. The findings illustrate the negative impact of *G. banksii* propagation on the regeneration and the conservation of native tree species in Madagascarian forest.

Keywords: *Grevillea banksii*, invasive plant, microbial community, native tree species, cluster roots.

1. Introduction

In recent years, naturalization and invasion of alien plant species and their impacts on the conservation of Madagascarian biodiversity have attracted the attention of many scientists [1, 2]. Indeed, the invasion of exotic species constitutes a serious threat to native ecosystems and economics [3], and is the second cause of biodiversity losses after habitat destruction [4].

Recent studies have demonstrated that introduced species encounter less inhibitory effects of soil biota where they are introduced than in their home range [5-7]. They have the ability to modify soil physico-chemical characteristics and to disrupt at the same time the regeneration of native species [8-11]. Since *G. banksii* dominates the forest land in eastern part of Madagascar, it was thought that this exotic species could create a new structure and functioning of soil microbial communities which become

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favorable for the propagation of the alien plant and inhibit the development of native plant species.

The main objective of this study is to describe the effects of *G. banksii* propagation on the structure and functioning of the soil mycorrhizal community and the symbiotic nitrogen-fixing bacteria on three plots where the vegetation cover is characterized respectively by grasslands, (P1), by homogeneous population of *G. banksii* (P2) and by natural forests of *Dalbergia trichocarpa* and *Intsia bijuga* (P3).

2. Materials and Methods

2.1. Field site Description

The study was conducted within relics of natural forest situated in eastern part of Madagascar (19° 07'S; 48° 54'E). This forest formation is largely surrounded by *G. banksii* where the small cluster of natural forest consists of mixed *D. trichocarpa* and *I. bijuga* native stands. Study areas were selected among the surface areas where little anthropogenic disturbance occurred during at least the last five years. Different plots were identified on the basis of plant composition including a mixed population of *D. trichocarpa* and *I. bijuga*, an invaded area by *G. banksii* and a control area at forest edge devoid of forest plant species.

2.2. Greenhouse experiment

Soil samples collected from each plot were sieved through a 2 mm mesh sieve and packed in 1 L plastic bags. Pots were arranged in a randomized complete block design with 30 replicates per soil type. Seeds of *G. banksii* were surface-sterilized by immersion in 70% ethanol and in sodium hypochlorite during 2 min and 20 min, respectively. They were then imbibed in sterile distilled water during 12 h and germinated on 1% agar. After 15 days of incubation at 30 °C, one pre-germinated seed was planted per pot. Seedlings were screened from the rain, grown under natural light (day length approximately 12 h, average daily temperature 25 °C) and watered three times a week with tap water during 4 months of culture.

A second experiment was conducted on the same soil types after 4 months of *G. banksii* growth. In order to assess the growth of *D. trichocarpa* and *I. bijuga* on each soil type precolonized by *G. banksii* and within or without this alien plant, the authors developed a device scheme according to which each native plant species was planted alone or accompanied by the alien plant. After 4 months, the growth of the native plant and functioning of soil microbial communities were assessed.

2.3 Laboratory analysis

2.3.1 Assessment of *G. banksii* development

After 4 months of culture, plants were harvested and the oven-dried mass (1 week at 65 °C) of shoot was measured. Their entire root system was washed under tap water and cluster roots per seedling of *G. banksii* were separated. The density of cluster roots was assessed by from the root system of each plant.

2.3.2 Mycorrhizal communities associated with each plant species

On each plant, the root system was gently washed, cleared and stained according to the method of Phillips and Hayman in 1970 [12]. The extent of mycorrhizal colonization was expressed as [*the number of mycorrhizal root pieces*]/[*total number of observed root pieces*] x 100. The number of root nodules per plant was determined.

Numbers of ectomycorrhizal roots and non-ectomycorrhizal roots were determined under a stereomicroscope (magnification x 60) for each root system to determine the percentage of ectomycorrhizal colonization (number of ectomycorrhizal short roots/total number of short roots). Remaining roots were oven-dried (1 week at 65 °C) and weighed.

2.3.3 Enzymatic activity of soils

Total microbial activity of each soil sample was measured before and after *G. banksii* cultivation by using the fluorescein diacetate (3', 6', -diacetylfluorescein [FDA]) hydrolysis assay

according to the method of Alef in 1998 [13]. This enzymatic conversion released a final product that can be determined colorimetrically at 490 nm, after 1 h of soil incubation. Total microbial activity was expressed as µg of hydrolysis product corrected for background fluorescence per hour and per gram of soil.

Phosphatase activity of rhizospheric soils of cluster roots and no cluster roots was measured in acid condition by absorbance readings at 400 nm following the method of Kuperman and Carreiro in 1979 [14] with p-NPP (p-Nitrophenol Phosphate) as a substrate of the enzymatic reaction and p-NP (p-Nitrophenol) as a final product.

2.3.4 Description of microbial population structure

Rhizospheric soil of each plant species was separated and dried at room temperature (25 °C). Number of total cultivable flora was assessed on Triptose Soy agar medium after multiple dilution of soil solution. Total number of cultivable flora was expressed as number of CFU (Colony Forming Unit) per g of dried soil.

2.4 Data analyses

Data were analyzed with one-way ANOVA. Means were compared using the Newman-Keuls test ($P < 0.05$). Percentages of the mycorrhizal colonization were transformed by arcsin (sqrt) before the statistical analysis.

3. Results

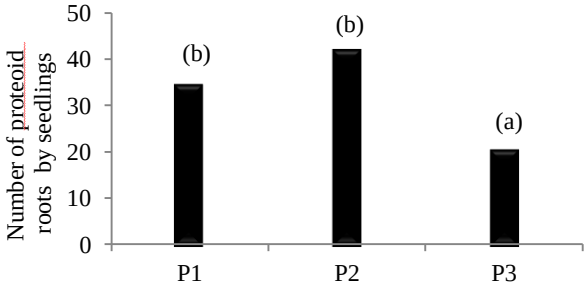
3.1 Development and description of the symbiotic status of G. banksii

After 4 months of development, seedlings of *G. banksii* were moderately endomycorrhized on the three types of soil. However, cluster roots were highly developed (Fig. 1) particularly on soils from P1 and P2 (Fig. 2).

Assessed by the shoot dry mass, the development of *G. banksii* was significantly higher on soil forest than on the two other soils where no significant difference was found (Fig. 3). However, seedlings of this alien

(a) (b)

Fig. 1 Roots system of *G. banksii* (a), cluster roots of *G. banksii* (b).



P1: Vegetation cover characterized by grasslands (Control)
P2: Homogeneous population of *G. banksii*
P3: Natural forest with the two native plant species

Fig. 2 Number of cluster roots by seedlings of *G. banksii* under 3 types of soil (Means followed by the same letter are not significantly different according to the Newman-Keuls test ($p < 0.05$).

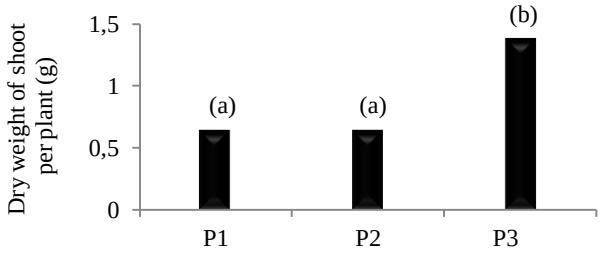
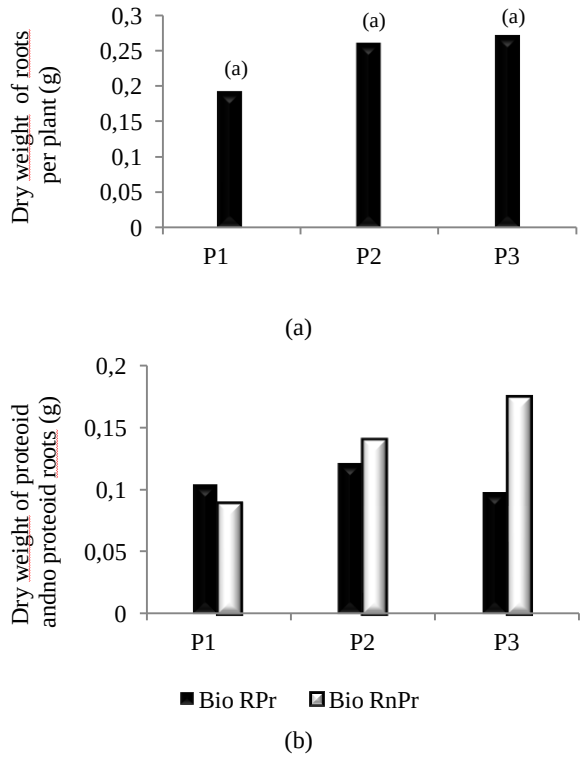


Fig. 3 Development of seedlings of *G. banksii* on three types of soil.

plant produced a similar root biomass on all three soil types (Fig. 4 a).

The proportion of biomass cluster roots and non-cluster roots per seedling of *G. banksii* varied moderately depending on soil type. It was almost the same level in P1 and P2. However, development of cluster roots was stimulated more than 2 times compared to no cluster roots on soil from P3 (Fig. 4

b).



Bio RPr: Biomass cluster roots

Bio RnPr: Biomass no cluster roots

Fig. 4 Root biomass of *G. banksii* on three soil types (a). Biomass of cluster roots and no cluster (b).

3.2 Soil microbial activities

After four months of soil colonization by *G. banksii*, total microbial activity has significantly decreased on all soil types. Between the three types of soil, the amount of fluorescein produced was significantly lower on the control soil (P1) compared to those observed on the other soil types (P2 and P3) (Fig. 5). Generally, the installation of the exotic plant strongly inhibited the total microbial activity.

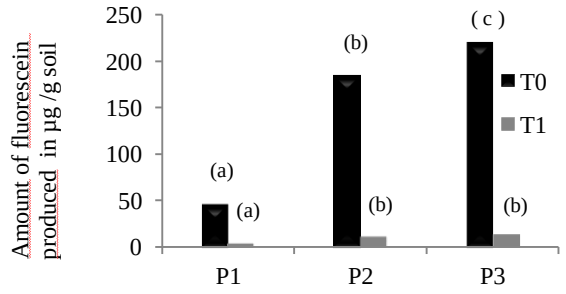


Fig. 5 Overall soil microbial activity (hydrolysis of fluorescein diacetate) before (To) and after the installation of *G. banksii* (T1).

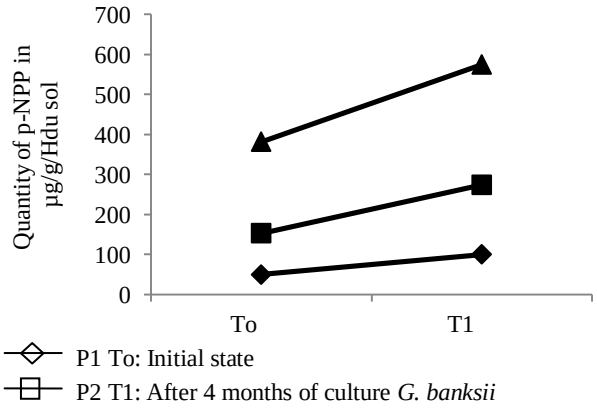
Soil phosphatase activity was significantly higher on the forest soil (P3) than that recorded in the other two soil types (Fig. 6). After four months of colonization by *G. banksii*, this activity phosphatase was generally stimulated in the three soil types (Fig. 6). This stimulation was significantly higher on the soil adhering with cluster roots of *G. banksii*.

3.3. Number of total cultivable microflora

At the beginning of the experiment, the number of total cultivable flora on each soil type was significantly lower on the forest soil compared to that recorded on the other two soil types (Fig. 7). The number of total cultivable microflora was not significantly modified by the colonization of land by *G. banksii* for the soil from colonized area by this plant and the control (Fig. 7). However, the propagation of this alien plant largely reduced the number of total cultivable microflora on soil from natural forest (P3).

3.4. Impacts of the soil colonisation by G. banksii on the regeneration D. trichocarpa and I. bijuga

The shoot and the root growth of the seedling, the number of ECM morphotypes, the number of nodule per plant and the AM colonization rate of *D. trichocarpa* were significantly reduced by the colonization of soil by *G. banksii* (Table 1). On soil originally from colonized area by this alien plant, no structure of vesicular or arbuscular mycorrhize was



P3

Fig. 6 Activity of soil phosphatase.

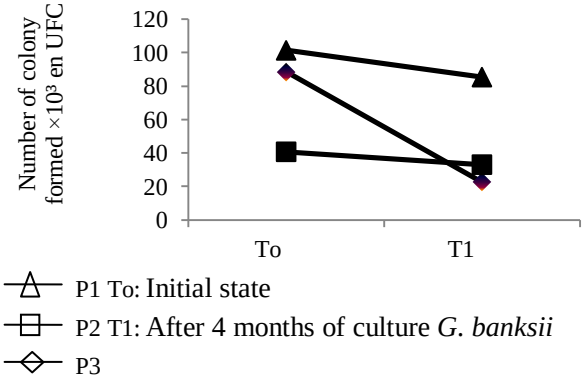


Fig. 7 Enumeration of total cultivable flora before (To) and after the installation of *G. banksii* (T1).

Table 1 Nodule number and mycorrhizal rate on root system of *Dalbergia trichocarpa* and *Intsia bijuga*.

	Number of nodules in *D. tricocarpa* (Nb / seedling)	Endomycorrhi-zation rates in *D. trichocarpa* (%)	Ectomycorrhi-zation rates in *I. bijuga* (%)
P1	5	25	37
P2	1	0	13
P3	5	50	69

found on root system of seedling. However, the soil colonization by *G. banksii* modified slightly the mycorrhzal rate (ECM and VAM) of seedling on forest soil (P3).

4. Discussion

The results of this study show that *G. banksii*, a plant species moderately mycotrophic, develops a high density of cluster roots in order to satisfy its nutrients needs on native ecosystems. The density of cluster roots varies considerably within the level of soil degradation and/or the composition of plant cover. The naturalization and invasion of this exotic plant has led to a high degree of disturbance on Malagasy forest ecosystems.

Some authors have already shown that development of invasive plants is better than that of native plant species in ecosystems with high nutrient availability [15-17]. On the first hand, the results of this study indicate that *G. banksii* grows within the forest landed in eastern part of Madagascar. On the other hand this exotic plant was also able to grow on degraded environment through the development of cluster roots that help the nutrition of the plant. This mechanism makes this alien plant more competitive than the native plants in low nutrient availability condition.

In addition, the propagation of the invasive plant induces profound changes in the development and the activity of soil microbial communities. It has been demonstrated that the disturbance can inhibit the development of soil symbiotic microorganism communities [18, 19]. The authors' results clearly demonstrate that *G. banksii* does disturb the development of these groups of soil microrganisms and the formation of symbiosis such as arbuscular mycorrhizal, ECM and nitrogen fixing symbioses with native plant species. These results corroborate the previous observations of Stinson et al. in 2006 [20] who documented that the invasive plant *Alliaria petiolata* inhibits the growth of native tree seedlings through interference with the soil biota.

5. Conclusions

The authors' results illustrate the pervasive characteristic of *G. banksii* by its ability to form cluster roots, especially in conditions of poor soil nutrients. These root types are surrounded by a high phosphatase activity. The development of these root types induces a strong disturbance within the functioning and the structure of symbiotic microflora communities and its associated microorganisms. This situation constitutes a real threat for the regeneration of Malagasy native plant species and the conservation of Madagascarian biodiversity.

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References

P. Binggeli, Introduced and Invasive Plants, In S.M. Goodman & J.P. Benstead (Eds.), The natural history of Madagascar, Chicago press, London, 2003, pp. 257-268.

S.M. Carrière, E. Randrianasolo, J. Hennenfent, Aires protégées et lutte contre les bioinvasions: des objectifs antagonistes?, Le cas de *Psidium cattleianum* Sabine (Myrtaceae) autour du parc national de Ranomafana, Vertigo 8 (2008) 1-14.

D. Pimentel, S. McNair, J. Janecka, J. Wightman, C. Simmonds, C.O'Connell, E. Wong, L. Russel, J. Zern, T. Aquino, T. Tsomondo, Economic and Environmental Threats of Alien Plant, Animal and Microbe Invasions, In: Pimentel D. (Eds.), CRC Press, London, 2002, pp. 307-329.

P.M. Vitousek, C.M. D'Antonio, L.L. Loope, M. Rejmanek, R. Westbrooks, Introduced species: A significant component of human-caused global change, New Zealand Journal of Ecology 21 (1997) 1-16.

R.M. Callaway, G.C. Thelen, A. Rodriguez, W.E. Holben, Soil biota and exotic plant invasion, Nature 427 (2004) 731-733.

J.L. Hierro, J.L. Maron, R.M. Callaway, A biogeographical approach to plant invasions: The importance of studying exotics in their introduced and native range, Journal of Ecology 93 (2005) 5-15.

R.H.A. Van Grunsven, W.H. van der Putten, T.M. Bezemer, F. Berendse, E.M. Veenendaal, Plant-soil interactions in the expansion and native range of a poleward shifting plant species, Glob Change Biol. 16 (2010) 380-385.

D. Pimentel, L. Lach, R. Zuniga, D. Morrison, Environmental and economic cost of non indigenous species in the United States, Bioscience 50 (2000) 53-65.

R.J. Cabin, S.G. Weller, D.H. Lorence, S. Cordell, L.J. Hadway, R. Montgomery, D. Goo, A. Urakami, Effects of light, alien grass and native species additions on Hawaiian dry forest restoration, Ecological Applications 12 (2002) 1595-1610.

CBD [Convention on Biological Diversity], Invasive Alien Species, Convention on Biological Diversity, (2006), Site Web: http://www.biodiv.org/programmes/cross- cutting/alien/.

S.J. Meiners, Apparent competition: An impact of exotic shrub invasion on tree regeneration, Biological Invasions 9 (2007) 849-855.

J.M. Phillips, D.S. Hayman, Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment if infections, Transactions of the British Mycological Society 55 (1970) 158-161.

K. Alef, Estimation of the Hydrolysis of Fluorescein Diacetate, In: Alef K., Nannipieri P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry, Academic Press, London, 1998, pp. 232-233.

R.G. Kuperman, M.M. Carreiro, Relationships between soil heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem, Soil Biol. Biochem. 29 (1979) 179-190.

M.J.W. Burke, J.P. Grime, An experimental study of plant community invisibility, Ecology 77 (1996) 776-790.

D.A. Wedin, D. Tilman. Influence of nitrogen loading and species composition on the carbon balance of grasslands, Science 274 (1996) 1720-1723.

J.S. Dukes, H.A Mooney, Does global change increase the success of biological invaders?, Trends in Ecology & Evolution 274 (1999) 135-139.

J.M. Vincent, A manual for the pratical study of root-nodule bacteria, International Biological Programme, Handbook N15, 1970, Blackwell, Oxford. UK.

S.E. Smith, D.J. Read, Mycorrhizal Symbiosis, 2nd edition, Academic Press, San Diego., 1997, p. 605.

K.A. Stinson, S.A. Campbell, J.R. Powell, B.E Wolfe, R.M. Callaway, G.C. Thelen, S.G. Hallett, D. Prati, J.N. Klironomos, Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms, Plos. Biol. 4 (2006) 727-731.