

Propagation of *Grevillea banksii*, an invasive exotic plant species: impacts on structure and functioning of mycorrhizal community associated with natives tree species in eastern part 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 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 structure of the vegetation are observed such as an area characterized by grassland (P1), by homogeneous population of *Grevillea banksii* (P2) and by a natural forest composed mainly by *Intsia bijuga* or *Dalbergia trichocarpa* (P3 and P4). Structure of mycorrhizal fungi communities and associated microorganisms were described on soils from each study plots. Cultivated on P1, P2, P3, P4 soils previously colonized by *Grevillea banksii* during 4 months, the development of *Intsia bijuga* and *Dalbergia trichocarpa*, was evaluated after 4 months culturing. According to the nutrients availability on each soil types, the development of *Grevillea banksii* was accompanied or not by a high formation of proteoid roots. Our results show also that soil occupation by *Grevillea banksii* decreased the total microbial and phosphatases activities of soil especially on soil within a high density of proteoid roots. Slightly mycotrophic, *Grevillea banksii* disturb 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 : Invasive plant, *Grevillea banksii*, microbial community, proteoid roots , native tree species

1 INTRODUCTION

In recent years, installation of alien plant species and their impacts on the conservation of Madagascarian biodiversity has attracted the attention of many scientists (Binggeli, 2003, Carrière et al., 2008). Indeed, the invasion of these species constitutes a serious threat to native ecosystems and economics (Pimentel 2002), and is the second cause of biodiversity loss after habitat destruction (Vitousek et al. 1997).

Recent studies have demonstrated that introduced species encounter less inhibitory effects of soil biota where they are introduced than in their home range (Callaway et al. 2004; Hierro et al. 2005; van Grunsven et al. 2010), have a ability to modify soil physico-chemical characteristics and to disrupt at the same time the regeneration of native species (Pimentel et al., 2000; Cabin et al. 2002; CBD, 2006; Meiners, 2007). Since *Grevillea 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 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 *Grevillea 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)

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and by natural forests of *Dalbergia trichocarpa* and *Intsia bijuga* (P3).

2 MATERIALS AND METHODS

2.1. Field site description

This 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 form mixed stands with *D. trichocarpa* and *I. bijuga*, two native forest species. 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 2mm mesh sieve and packed in 1L 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-pregerminated seed was planted per pot. Plants 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 development of *G. banksii*. In order to assess the development of *D. trichocarpa* and *I. bijuga* on each soil type precolonized by *G. banksii* and within or without this alien plant, we developed a device scheme according to which each native plant species was planted alone or accompanied by the alien plant. After 4 months of growth, development of native plant was assessed and functioning of soil microbial communities was described.

2.3 Laboratory analysis

2.3.1 Assessment of *G. banksii* development

After 4 months of culture, 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 and proteoid roots per seedling of *G.*

banksii were separated. The concentration of proteoid root was assessed by root system of each plant.

2.3.2 Mycorrhizal communities associated with each plant species

On each plant, the root systems were gently washed, cleared and stained according to the method of Phillips & Hayman (1970). The extent of mycorrhizal colonization was expressed as [the number of mycorrhizal root pieces]/[total number of observed root pieces]x100. The number of root nodule per plant was determined.

Numbers of ectomycorrhizal roots and non-ectomycorrhizal roots were determined under a stereomicroscope (magnification x 60) for each lateral root 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 (1998). 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 soils from adhering of proteoid root and no proteoid root was measured in acid condition by absorbance readings at 400nm following the method of Kuperman & Carreiro (1997) with p-Nitrophenol Phosphate (p-NPP) as a substrate of the enzymatic reaction and p-Nitrophenol (p-NP) 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 Tryptose Soy agar medium after multiple dilution of soil solution. Total number of cultivable flora was expressed as number of Colony Forming Unit (CFU) per g of dried soil.

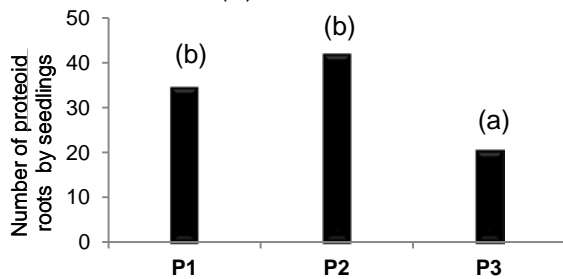
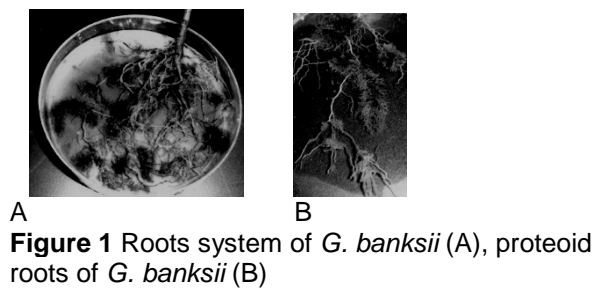
2.4 Data analyses

Data were treated 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 RESULTATS

3.1 Development and description of the symbiotic status of *Grevillea banksii*

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



P1: Vegetation cover characterized by grasslands (Control)

P2: Homogeneous population of *G. banksii*

P3: Natural forest with the two native plant species

Figure 2 Number of proteoid roots by seedlings of *G. banksii* under 3 types of soil.

Assessed by the dry weight of shoot, the Development of *G. banksii* was significantly high on soil forest than on the two others soils where no significant difference was found (Fig 3). However, seedlings of this alien plant produce a similar root biomass on all three soil types (Fig.4A).

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

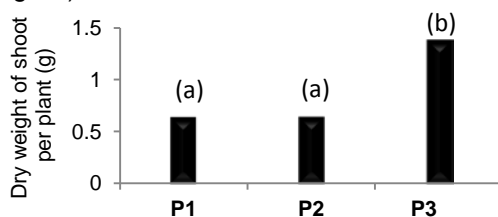
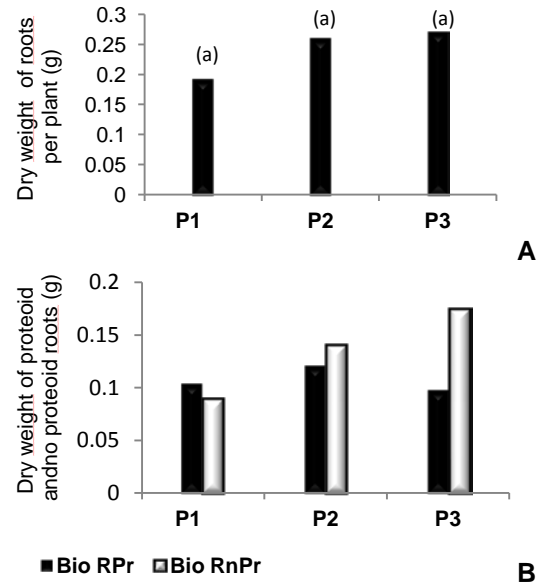


Figure 3 Development of seedlings of *G. banksii* on three types of soil.



Bio RPr: Biomass proteoid roots
Bio RnPr: Biomass no proteoid roots

Figure 4 Root biomass of *G. banksii* on three soil types (A) Biomass of proteoid roots and no proteoid (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 produced fluorescein was significantly low 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 was strongly inhibited the total microbial activity.

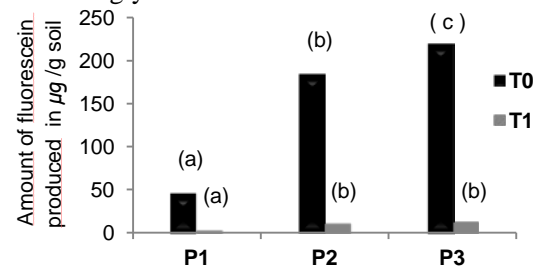
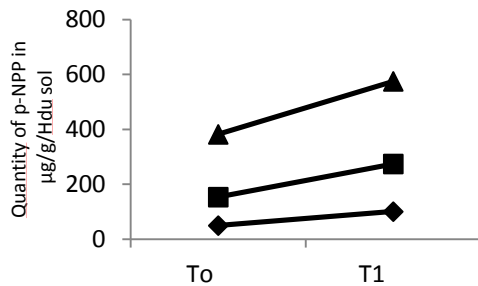


Figure 5 Overall soil microbial activity (hydrolysis of fluorescein diacetate) before (T0) and after (T1) the installation of *G. banksii*

Soil phosphatase activity was significantly higher on the forest soil (P3) than those recorded in the other two soils 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 significant high on the soil adhering with proteoid roots of *G. banksii*

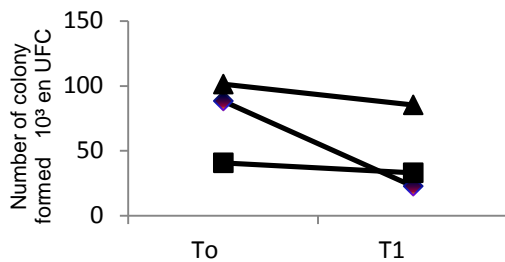


- ◇ P1 To: Initial state
- P2 T1: After 4 months of culture *G. banksii*
- △ P3

Fig.6 Activity of soil phosphatase

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 low on the forest soil compared to those 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 has largely reduced the number of total cultivable microflora on soil from natural forest (P3).



- △ P1 To: Initial state
- P2 T1: After 4 months of culture *G. banksii*
- ◇ P3

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

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

Table 1. Nodule number and f mycorrhizal rate on root system of *D. trichocarpa* and *I. bijuga*

	Number of nodules in <i>D. trichocarpa</i> (Nb / seedling)	Endomycorrhization rates in <i>D. trichocarpa</i> (%)	Ectomycorrhization rates in <i>I. bijuga</i> (%)
P1	5	25	37
P2	1	0	13
P3	5	50	69

4 DISCUSSION

The results of this study show that *G. banksii*, a plant species slightly mycotrophic, develop a high density of proteoid root in order to satisfy its nutrients needs on native ecosystems. The density of proteoid roots varies considerably within the level of soil degradation and or the composition of plant cover. The installation of this exotic plant led to high degree of disturbance on Malagasy forest ecosystems.

Some authors have already illustrated that development of invasive plants is better than those the native plant species in ecosystems with high nutrient availability (Burke & Grime, 1996; Wedin & Tilman 1996; Dukes & Mooney, 1999). In the first hand, the results of this study indicate that *G. banksii* grows within the forest land in eastern part of Madagascar. On the other hand this exotic plant was also able to grow on degraded environment through the development of proteoid roots that support the nutrition of the plant. This mechanism transforms the alien plant to be more competitive than the native plant 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 within the soil symbiotic microorganism communities inhibit the development of these symbionts (Vincent, 1970, Smith & Read, 1997). Ours results clearly demonstrate that *G. banksii* disturb the development of these groups of soil microorganisms and the formation of symbiosis such as vesicular or arbuscular mycorrhize, ECM and nitrogen fixing symbiosis with native plant species. These results corroborate the previous observations of Stinson et al. (2006) who documented that the invasive plant, *Alliaria petiolata*, inhibit the growth of native tree seedlings through interference with soil biota.

5 CONCLUSION

Our results illustrate the pervasive characteristic of *G. banksii* by its ability to form proteoid 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 constitute a real threat for the regeneration of Malagasy native plant species and the conservation of Madagascarian biodiversity

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