



Seed Bank and Seedling Recruitment Following Ten-Year Cessation of Cattle Pastures in Serrania De Los Yariguies National Park

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Abstract

Seed bank and seedling recruitment following cessation of cattle pastures is a neuralgic topic to clarify the natural regeneration in a tropical forest. Land-use changes are related to the reduction in tree density and biomass, as well as changes in the vegetation distribution. A little-studied consequence is an effect on the soil seed bank and seedling recruitment, which are essential for natural forest regeneration. Natural regeneration starts the ecological succession without human intervention; otherwise, the active ecological restoration will be necessary for supporting the process. Here, we were focused on the effects of ferns and abandoned pasture vegetation on tropical rain forest regeneration. We assumed that the presence of invasive species in pastures delays the recovery time of plant communities after a disturbance. In Serranía Los Yariguíes National Park in Golconda locality, exotic pastures and ferns are the typical covers in abandoned cattle pastures. We carried out seed germination assays and field experiments involving cover clearing to evaluate the effect of soil cover on the soil seed bank and seedling recruitment. Our results showed that ferns and pastures are a factor in arresting natural forest regeneration. However, in soils with fern cover, the seed bank is a source of propagules to give continuity to the ecological succession. The creation of exclusion's micro-sites in pasture and fern showed a significant effect on the recruitment of seedlings. The response of the soil seed bank and seedling recruitment is essential in defining the more cost-effective ecological restoration activities and support the management decisions.

Keywords: Ferns, Natural regeneration, Pasture, Secondary forest, Seed bank, Seedling recruitment

Introduction

Soil-use change to cattle pasture and agriculture are the top activities causing transformation and loss of the tropical forest [1]. The consequences of these activities include habitat loss and biodiversity reduction [2-5]. Also, the transformed areas are susceptible to invasive species' arrival and establishment Gurrutxaga, Lozano [6], which limits the native plant community's recovery after the human activity has ceased [1,2]. Invasive species change the soil conditions, promoting fertility loss, organic matter quantity and quality reduction, changes in the soil's pH, and alterations in the soil's cationic change capacity [7]. In the biological framework, invasive species reduce the soil potential for the native seed store, seed germination, and seedling establishment, with a direct effect on the native plant community's recovery [2]. Such

effects are the consequence of the high seeds production, high germination and survival rates, and high relative growth rates that define the invasive species' behavior. Besides, invasive species display traits and biomass allocation strategies that give them an advantage in environmental adaptation over the native tree species in the absence of natural enemies. *Brachiaria* sp. and *Urochloa decumbens* (Stapf) R.D. Webster are African species typically introduced to cattle pastures [8]. What happens to such pastures after they are abandoned has attracted particular interest when it comes to an understanding of the natural regeneration processes, as well as conducting experimentation in restoration ecology and the practices of ecological restoration. Prior results have shown the extreme environmental conditions associated with high solar radiation reducing native species' capacity to become established,

as well as their rates of photosynthesis and growth [9]. Direct sun exposure increases the soil temperature and evaporation, making seeds and seedlings likelier to become dehydrated [9]. An additional stressor is the presence of ferns, especially those of the *Pteridium* genus, which are strong competitors in cattle pastures. Ferns are faster colonizers in areas exposed to burning, which is a common practice in cattle pasture management [9-11]. Ferns prevent the seedling establishment, deplete the soil seed bank (SB), and impede secondary succession [12].

Moreover, ferns develop a dense root layer with underground rhizomes that have high reserves of carbohydrates and nutrients, as well as regenerative parts that produce new fronds. The aboveground compartment is a dense dosel, which favors shadow and litter accumulation; the litter's chemical composition repels other species, thereby preventing their colonization [9,13]. In fern areas, the remnant trees are scarce, and the natural regeneration is reduced. However, some tree species can become established, and previous results have shown that the litter layer favors seedlings by replacing herbs and pastures [9]. In areas with invasive plant species, the physical and biotic characteristics of the affected area must allow for the recruitment and germination of native plant species to assist in the plant community's recovery [1]. The availability of local resources, such as soil nutrients, humidity, the presence of microorganisms, and the availability of propagules, are essential in the regeneration of the forest. The soil SB, defined as all living seeds in a soil profile, including those on the soil surface Saatkamp [14], is the central reserve of plant propagules Chazdon, Guariguata, Martínez & Uriarte Chazdon [15], and it determines the opportunity for natural regeneration. The SB reduces the possibility of population extinction and promotes species coexistence; in some cases, it is the most important source of plants after a disturbance [16-19]. After the disturbance, the plant regrowth is affected by propagule and resource availability [15]. The competition with pastures and ferns and the physical conditions can strongly affect the composition and rate of forest regeneration [20].

The SB depends on the climate, herbivory, and disturbance. In pastures surrounded by forest areas, a small group of plant species present in the forest reaches the interior of the pasture, mainly species dispersed by animals, while other species dispersed by the wind are usually abundant. Due to the low availability of seeds in the pastures, the recovery of tree vegetation is slow, with low diversity earlier in succession [21]. Advanced successional forests in pastures have small SBs (in density and composition), as the seeds of shade-

tolerant species (primary, late pioneer) enter a smaller proportion and produce a transient SB (Garwood 1989). Nonetheless, the persistence of pastures and the presence of higher extensions of ferns make it necessary to identify the appropriate mechanisms that will allow the recovery of degraded areas; thereby, improving the conservation status in Serranía de Los Yarigués National Park and serving as a reference to improve the connectivity with surrounding areas. The main objective of the present study was to determine whether the soil SB and seedling recruitment differ in two types of soil cover in an abandoned pasture in a natural park in northeast Colombia. Also, we hoped to determine whether a single cutting (i.e., a single moment of pruning) of the pasture and ferns would represent an effective strategy for accelerating the natural regeneration processes. We expect the SB in areas with invasive species, whether ferns or pastures, will show lower germination. Also, we expect a simple cutting of ferns and pastures will increase the seedling recruitment in the experimental areas.

Materials and Methods

Study area

The study was conducted in the Golconda locality (06°35'33.4" N; 73°21'15.8" W) in Serranía de Los Yarigués National Park, which was declared a protected area in 2005 (Document N° 603, May 13). Yarigués Park is in northeast Colombia, in the western foothills of the Eastern Andean Range, 2100 m above mean sea level. It is part of the Tropical Montane Rain Forest life zone (Holdridge 1996), and it has a mean annual precipitation of 2000 to 3000 mm in a bimodal regime, with peak levels of rainfall occurring both in April–May, and September–October. The mean annual temperature is 16.7°C [22]. Table 1 summarizes details concerning the soil characteristics across the studied sites. The study area was previously used for corn, soy, banana, and yuca (*Zea miz Vell.*, *Glycine max (L.) Merr.*, *Musa × paradisiaca L.* and *Manihot esculenta Crantz*, respectively) cultivation. Posterior to cultivation the area was employed for cattle pasture; areas show loss of native vegetation and an increase of ferns (*Pteridium aquilinum (L.) Kuhn* and *P. arachnoideum (Kaulf.) Maxon*) and grasses [23]. Cattle pasture management included fire for increasing the pasture growth and herbicide application to avoid native species propagation. Forest in the study site has been affected by cattle pasture and fragmentation, with area reduction. The sites selected as reference were abandoned 60 years ago and have not been used for agriculture or grazing in recent history. The forest in the study area is a secondary forest dominated by tree species of the families Melastomataceae, Rubiaceae, Lauraceae, and Araliaceae.

Table 1: The mean and standard error of physical and chemical values of soil in the study sites into the Natural Park Serrania de Los Yariquíes, location Golconda. N = 27. **P < 0.01, ***P < 0.001. Different lowercase letters indicate means are significantly different (P < 0.05) among soil covers.

	Fern Area	Pasture	Secondary Forest	F
Nitrogen (mg/kg)	4200(552.7) ^b	5566(424.5) ^b	9255(1410.4) ^a	0.0074 **
Phosphorus (mg/kg)	6.07(1.53) ^b	3.09(0.44) ^b	12.55(1.94) ^a	4.41e-05***
pH	5.5 (0.2)	5.8 (0.03)	5.7 (0.03)	NS
Soil Compaction (g cm ²)	31 (1.53) ^a	27.02 (1.53) ^b	28.65 (4.56) ^b	0.0079**
Soil Density (Mg m ³)	1.0(0.02) ^b	1.4(0.01) ^a	0.92(0.03) ^b	0.0001**
Soil Texture (%)				
Sand	36.0 (4.2) ^a	27.8(2.3) ^c	32.1(2.4) ^b	0.045*
Silt	12.6(3.1) ^b	17.0(1.7) ^a	14.5(1.9) ^b	0.024*
Clays	49.7(6.1) ^a	54.2(1.6) ^a	56.2(2.3) ^a	2.4NS

Experimental Design

To select areas, we considered the similarity in the soil use history and the time since abandonment; besides, we select sites into the same landscape matrix. We selected three soil covers, secondary tropical forest (Fo), pasture (P), and *P. aquilinum* (Pa). Forest is an intermedia successional forest according to local expert knowledge of specific site history. In each soil cover, we selected three sites and installed three replicated 50 m² (10 × 5 m) plots per site. Plots in Fo were located 100 m inside from the forest border. In P, we located plots taking at least 100 m outside from the forest border. Plots in Pa were located to 100 m inside from the border of the *P. aquilinum* cover. In each plot, we installed eight quadrants (1 m²) for a total of 24 quadrants. In four quadrants per plot, herbaceous vegetation was cleared (i.e., herbs and shrubs in Fo, grass in P, and fern in Pa cover); the four remaining quadrants per plot were maintained with the original soil cover as control quadrants. After that, we will refer to treatments as cleared and un-cleared quadrants.

Seed Bank and Seedling Recruitment

During the dry season (December 2016), we collected four soil cores from the topsoil (10 cm depth) of each plot using a soil sampler of 7.5 cm in diameter. The litter layer was removed before the soil collection. The soil samples were stored in plastic bags until the process for the seed germination assay. The samples were homogenized and distributed in a 400-cm² plate with a perforated bottom for water drainage. We used soil for all plates to reach 2.5 cm depth, and all plates were kept in a shady greenhouse. The light and humidity conditions were similar for all plates in the greenhouse. Seedling emergence was monitored every month for six months. The seedling emergence in plates was used to estimate soil SB density and morphospecies richness. Because we were focused on the soil SB potential to recover the forest, we included tree-seedling only. In the field, we registered individual recruitment in both treatments, cleared and un-cleared quadrants, from November

2016 to August 2017. We registered seedling density (i.e., the individual number per quadrant) and morpho-species richness. To include an individual into the register, we selected this difference of grasses and ferns.

Data Analysis

The seedlings emergence in soil from the three soil covers were compared using a generalized linear model with Poisson distribution, employing the number of plants emerging in the plates. To test the effect of soil cover and treatments (i.e., cleared, or un-cleared) on the seedling recruitment, we carried out a generalized linear model with Poisson distribution, the interaction between the two factors was included. To compare the rates of seed germination and plant recruitment across the three soil covers (and cleared or un-cleared treatment), an analysis of covariance (ANCOVA) was carried out. All analyses were performed using R software, version 2.15.2.

Results

Soil Seed Bank

The total number of germinated seeds, considering all the soil cover plates, was 3415 in 108 soil samples. The seed germination census began 30 days after the soil preparation and continued to 194 days. We found higher seed germination in soil from *P. aquilinum* cover (1487 seedlings), followed by the soil from the forest (1073 seedlings) and pasture (855 seedlings). The rates of seed germination were different across the three soil covers ($Z = 71.57$; $p = 0.001$). The soil SB from the fern cover showed the highest germination rate (34.69 ± 1.20 seeds per day), followed by the soil SBs from the secondary forest (23.42 ± 1.25) and pasture (21.29 ± 1.02 ; Figure 1). The number of morpho-species was affected by the soil cover ($Z = 14.64$; $p = 0.001$). The richness of morpho-species was higher in *P. aquilinum* (4.97 ± 0.25), followed by forest (3.61 ± 0.26) and pasture (2.83 ± 0.20).

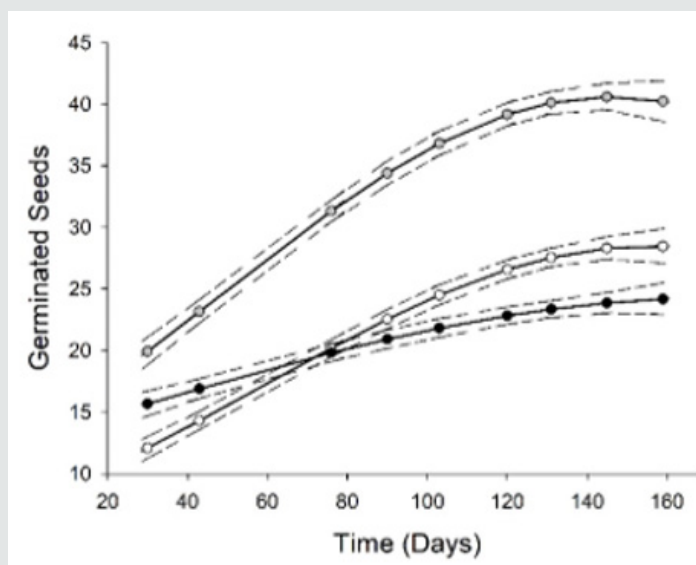


Figure 1: Germination time course and 95% confidence interval bands in the plate experiment. Soil from the forest (empty circle), *P. aquilinum* (gray circles), and pasture (dark circles).

Seedling Recruitment

The seedling recruitment census began 57 days after the plots were installed, and it continued for 284 days. There were 16416 seedlings counted in the sampled plots across the three soil covers and two treatments. The soil cover significantly affected the number of seedlings ($Z = 176.37$; $p = 0.0001$). Most seedlings were recruited in the *P. aquilinum* quadrants (29.36 ± 3.06), followed by the secondary forest (17.77 ± 3.05) and pasture (17.52 ± 3.94). In the same way, the treatment affected the seedling recruitment count ($Z = 72.55$; $p = 0.001$). The greatest seedling recruitment was found in the cleared plots (19.90 ± 1.31) in comparison with un-cleared ones (12.13 ± 1.33 ; Figure 2). In the cleared plots, there

were 10750 seedlings recruited counted, and in un-cleared plots, 6551 plants across the three soil covers. The seedling recruitment across the soil covers varied per treatment ($Z = 72.55$; $p = 0.001$). In the cleared sites, the plots in the pasture showed the biggest seedling recruitment rates (33.80 ± 2.48), followed by *P. aquilinum* (18.56 ± 1.95) and forest (7.35 ± 1.74). In the un-cleared plots, the secondary forest plots showed the greatest number of seedlings (18.8 ± 1.46), followed by *P. aquilinum* cover (14.5 ± 1.06) and pasture (13.43 ± 1.60). The morpho-species richness was affected by the soil cover and treatment ($Z = 176$, $p = 0.0001$; $Z = 12.74$, $p = 0.001$, respectively). The greatest number found was in the morpho-species number in *P. aquilinum* sites (4.97 ± 0.22), followed by the forest (3.58 ± 0.13) and pasture (0.50 ± 0.11).

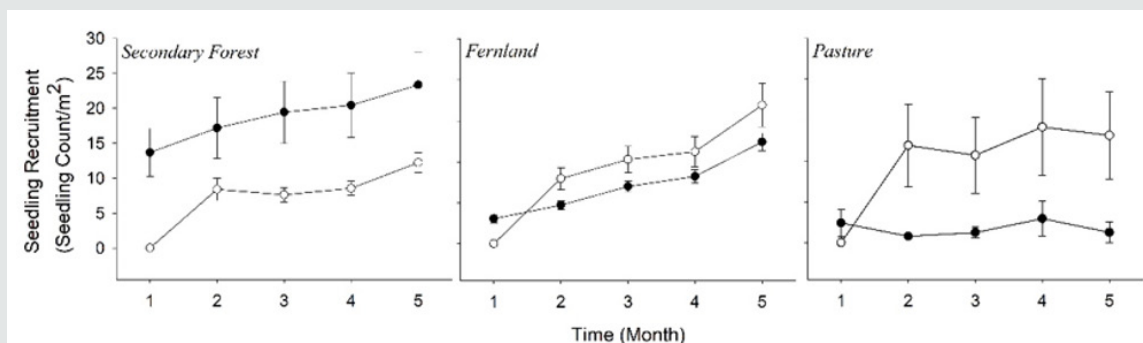


Figure 2: The mean and standard error of seedling recruited in un-cleared (dark circles) and cleared (empty circles) in secondary forest, fern lands and pasture. The error bars indicate the standard error value.

Discussion

The traditional soil used in the study sites with *P. aquilinum* and pasture cover could be the factor generating the decrease of the soil's nitrogen and phosphorus, as well as the increase in soil

compaction beneath *P. aquilinum* and soil density beneath the pasture. In contrast to our expectations, the seed density and diversity of morpho-species in the SB from *P. aquilinum* areas were higher than those found in the secondary forest. These results

suggest that *P. aquilinum* does not limit the incorporation of seeds into the soil, in agreement with the results reported by Xavier et al. (2016) and Ghorbani et al. (2006). In our study area, the ferns cover comprised of *P. aquilinum* and *P. arachnoideum*, which have been shown to limit productive practices and biodiversity conservation actions because they prevent seed germination and seedling establishment and growth [11,24-26]. However, the seedling abundance and morpho-species richness in the soil from *P. aquilinum* areas allowed us to consider that the ferns were acting in favor of the seed input and preventing seed predation in Golconda locality. The lower seed germination in soil from the secondary forest could have resulted from a limited seed input of pioneer species that colonized in the early stages of secondary succession (Guevara et al. 2005). Also, since the soil was sampled in locations inside the forest, far away from the border, the lower density of individual recruitment may have been a result of higher rates of seed predation [27]. The results found in the pasture were in concordance with our expectations and other reported findings (Xavier et al. 2016). The low germination, as well as the lower number of morpho-species, was evidence of the highly competitive power of the pasture species, such as *U. decumbens*, limiting the vegetation establishment and SB formation. The low seed density in the pasture has been associated with low seed rain and high seed and seedling predation (Esquivel et al. 2008; García-Orth & Martínez-Ramos 2008); the additional evidence of the apparent differences in the seedling recruitment in the presence of exotic pastures (in the un-cleared treatment) reinforces the theory about pasture's invasive power. Although we did not focus on the mechanisms for this, there is agreement on the role of pastures as physical barriers that limit the seeds' incorporation into the soil; they also favor high seed and seedling predation, and they are strong competitors for soil nutrients, water, and/or light [2,28,29]. These mechanisms may have governed the results found in the Golconda locality. Also, although it has been reported that seed rain in pastures declines with distance to the remnant forest, our studied pastures were at 10–25 m from the remnant forest, thereby falling within the limit for seed dispersion reported in the literature (Martínez-Garza & González-Montagut 1999; Cubiña & Aide 2001). We can expect the low seedling recruitment of the SB in the pasture was due to seed predation; though, we need more evidence to support this. Although our study has limited power for elucidating the mechanisms controlling such patterns, our results are in line with those reported on the SB dynamics in tropical forests. Our results highlight that actions favoring natural regeneration in pasture areas will require the enrichment of the SB.

The experimental site with a single cutting of exotic vegetation showed this was an effective treatment. The results indicated that, in the tropical rainforest of the National Park Serranía de Los Yariguies, pasture and *P. aquilinum* control represents a useful

strategy for the development of a plant community. We also showed the high potential for natural regeneration of fern cover, despite its high invasive power. Most emergence of seedlings in sites where ferns and pasture were cut once and germination assays in soil from ferns show us that fern areas represent a considerable contribution to the natural regeneration process in the transformed forest. In the field, the creation of exclusion micro-sites is stimulating the seedling recruitment, which contributes to the recovery of the diversity and species richness in concordance with the pattern reported in the literature [30,31]. Finally, we considered low seedling emergence in cleared tropical secondary forest as a consequence of slow dynamics, associated with low light availability, and possibly, an inhibition effect by species present in the SB. In conclusion, ten years after the land was abandoned, the effect of the productive activities carried out in the Golconda locality has been shown and continued to diminish the potential of the forest regeneration in favor of the presence of invasive species. Also, we can affirm that it is necessary to implement different management actions to achieve forest restoration. In fern areas, although ferns are challenging to eradicate, it is possible to take advantage of the availability of propagules to implement active restoration actions. In the meantime, it is necessary to implement active restoration strategies, such as the planting of vegetation nuclei with species native to the area. With this study, we can suggest the elimination of vegetation cover is a practice that can facilitate natural regeneration, especially for ferns, where there was a higher quantity of proposals and a greater diversity of species after clearing. Finally, there is an obvious need to carry out further work to ascertain the mechanisms governing the plant community's development in pasture and fern areas. The question is vital in protected areas where there is the potential to reverse the effect of the historical damage to the native plant community [32-34].

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