Edaphic and climatic control of macaúba palm seed bank dynamics

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ABSTRACT

The control of soil seed bank (SSB) dynamics is crucial to the ecologies of plant populations of many species, but has been little studied in tropical environments. The objectives of this work were to characterize the SSB of *Acrocomia aculeata*, an important neotropical oleaginous palm, and to define the influence of edaphic and climatic factors on its dynamics. Exposed and buried diaspores were collected in five areas of the Cerrado biome in central Brazil. Evaluations of the diaspores, young plants, and *in vitro* embryo cultures defined 26 indicators of SSB dynamics that were correlated with 19 physical-chemical attributes of the soil. Seedlings emergence was studied under greenhouse conditions during five years and the thermal control of germination was evaluated experimentally. *A. aculeata* forms persistent SSBs, with P being the main edaphic factor associated with seed input. High levels of Al and clay (associated with insect predation), organic matter (fungal contamination), and sand (bacterial contamination) contributed to negative outputs (embryo death). The maintenance of intact SSB embryos, however, is independent of edaphic factors. Wide thermal amplitudes in the winter associated with high temperatures in the spring promote overcoming dormancy and favor positive SSB outputs as well as the synchronization of seedling emergence at the beginning of rainy season. The high productivity of *A. aculeata* and its ability to maintain viable but dormant embryos in the soil contribute to controlling SSB dynamics, reproductive success, and its wide distribution.

1. Introduction

Soil seed bank (SSB) represent stocks of viable seeds in the soil that have crucial roles in the species regeneration and management of many plant populations (Thompson and Grime, 1979; Baskin and Baskin, 2014). Soil seed bank dynamics vary greatly among species and between ecosystems (Thompson et al., 1998; Csontos and Tamás, 2003), however, in-depth studies involving field and long-term SSB assessments in highly diverse tropical regions are just emerging (Long et al., 2014).

The persistence of SSB seeds precedes one of two possible fates: germination or death. Those outcomes are determined by characteristics of the seeds and how they are affected by external biotic and abiotic factors (Orozco-Segovia et al., 2003; Brock, 2011; Long et al., 2014; Jaganathan et al., 2019). Positive SSB outputs, which translate to seedling establishment, depend on specific seed adaptations to the risks and opportunities in their environments, and involve resistance to predation and/or microbial deterioration (resistance structures and mechanisms that preserve their longevity), as well as adjustment to

climatic conditions (germination control) (Scariot, 1998; Dalling et al., 2011; Long et al., 2014). The great diversity of climatic and edaphic (abiotic and biotic) interactions with SSB have limited the elucidation of the mechanisms controlling its dynamics –which will necessitate detailed field studies (using complementary methodologies) and long-term evaluations (Weiss, 1984; Wagner and Mitschunas, 2008; Gorecki et al., 2012; Long et al., 2014).

Palms are distributed pantropically, with neotropical rainforests harboring the greatest diversity (Dransfield et al., 2008; Orozco-Segovia et al., 2003). Most palms species in Brazil occur in the humid Amazonian and Atlantic Forest biomes (Lorenzi et al., 2010). Some species, however, show remarkable adaptations to seasonal environments with irregular precipitation, such as the Cerrado (neotropical savanna) biome, and their survival involves a series of physiological mechanisms and strategies that are still poorly understood (Ribeiro et al., 2011, 2012a; Neves et al., 2013; Oliveira et al., 2013; Carvalho et al., 2015).

Acrocomia aculeata (Jacq.) Lodd. ex Mart. (the macaúba or macaw palm) is widely distributed in the tropical Americas, with significant occurrences in Brazil (Fig. 1) (Scariot et al., 1991; Lorenzi, 2006;

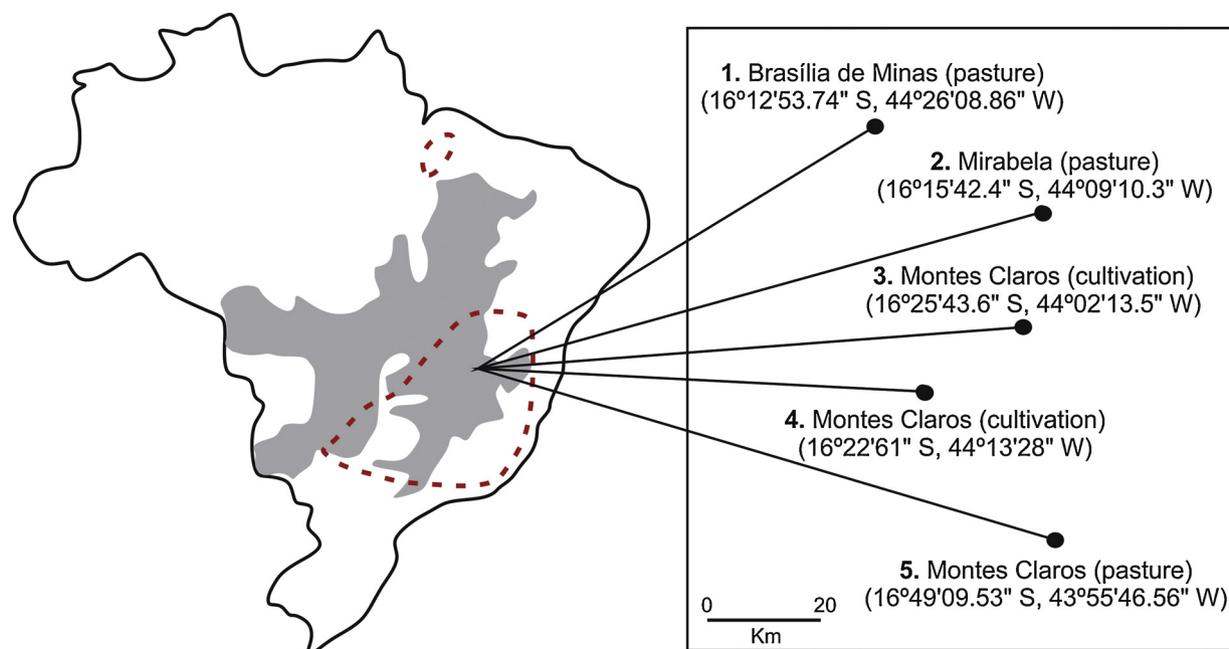**Fig. 1.** Map of Brazil, indicating the location and use characteristics of the study areas. The gray area represents the Cerrado biome; the dashed areas indicate regions where *Acrocomia aculeata* occurs.

Lorenzi et al., 2010). The species demonstrates an exceptionally high potential for biofuel production, especially in dry tropical regions, due to its high oil content (stored in the mesocarps and seeds) and its tolerance to water deficits (Pires et al., 2013). Macaúba plantations are still incipient, although the prospect of planting them on an industrial scale risks over exploding natural populations and highlights the importance of studies on their ecology as a basis for their domestication and sustainable harvesting (Lorenzi, 2006; Ribeiro et al., 2011; Pires et al., 2013). Additionally, there are silvopastoral enrichment and management proposals under consideration involving planting extensive areas of degraded pastures with *A. aculeata* (Cardoso et al., 2017) that will require detailed studies of their natural reproduction.

Macaúba seeds are orthodox (*i.e.*, desiccation and low temperature tolerant), and often require years to germinate due to their pronounced dormancy – which suggests the capacity to maintain persistent SSBs (Ribeiro et al., 2011, 2012a). However, there are no field studies to show the germination ecology of *A. aculeata* seeds and whether or not seeds can persist in the soil and establish SSB. The pronounced dormancy (classified as morphophysiological by Baskin and Baskin, 2014 and related to the complex structure of the diaspores, Fig. 3a and b) not allow a direct association between germinability and viability, and make it difficult to evaluate *A. aculeata* seed quality. The use of *in vitro* embryo culture, however, which can overcome dormancy and allow vigor estimations (Ribeiro et al., 2010; 2012a, 2012b) and long-term germination assessments, could contribute to the expansion of our knowledge about its SSB and reproductive strategies.

The objective of the present work was therefore to characterize the SSB of *A. aculeata,* define the roles of edaphic and climatic factors in its dynamics, and address the following questions: i) What is the classification of its SSB persistence? ii) How do soil physical and chemical factors influence SSB dynamics? iii) What are the roles of climatic factors in SSB output in terms of successful germination? We also sought to discuss the role of the SSB in the adaptation of the species to the environments in which it naturally occurs and the perspectives for the agricultural management of natural populations.

2. Materials and methods

2.1. Study areas

Five populations of *A. aculeata* (comprising more than 100 individuals each) were selected in the Cerrado biome in northern Minas Gerais State, Brazil (Fig. 1). The collection areas are distributed in three neighboring municipalities with climate classified as Aw, according to the Köppen and Geiger and similar average annual temperature and precipitation: Montes Claros (22.7 °C, 1029 mm), Mirabela (21.8 °C, 1074 mm), and Brasilia de Minas (22.2 °C, 1105 mm) (Inmet and Instituto Nacional de Meteorologia, 2018). All of the environments in the collection areas had been anthropized (for different uses), although they showed characteristics typically associated with the occurrence of macaúba palm trees.

2.2. Collection and preliminary evaluations

Ten fruiting plants in each population were arbitrarily selected. Fifty freshly dispersed fruits (their yellowish abscission scar was taken as an indicator of recent abscission - Carvalho et al., 2015) were collected from the ground in four quadrants established around each plant, 3–m from the stipe (Long et al., 2009). For SSB evaluation, areas (0.25 m²) were demarcated in the same quadrants and previously dispersed diaspores (fruits and pyrenes) exposed on the soil were collected (Fig. 2). Excavations (up to 20–cm deep) were made in the demarcated areas; all soil was removed and all buried diaspores were collected (in addition to approximately 500 g of soil).

Embryos obtained from the freshly dispersed fruits were cultured *in vitro* and their viability and vigor were evaluated. To that end, the fruits were opened (using a manual bench vise) and the embryos (Fig. 3a and b) removed and inoculated (under aseptic conditions in a laminar flow chamber) into test tubes (12 × 1 cm) containing 2 mL of MS medium at 75% of its original concentration: 0.4 mg/L thiamin; 1 mg/L pyridoxine; 0.5 mg/L nicotinic acid; 100 mg/L myo-inositol; 0.5 g/L hydrolyzed casein; 3 g/L activated charcoal; 30 g/L sucrose; 6 g/L agar; the pH was subsequently adjusted to 5.7. (Ribeiro et al., 2012b). The embryos were kept in germination chambers in the dark, at 30 °C. After 30 days of cultivation, the embryos and seedlings were evaluated. Embryos
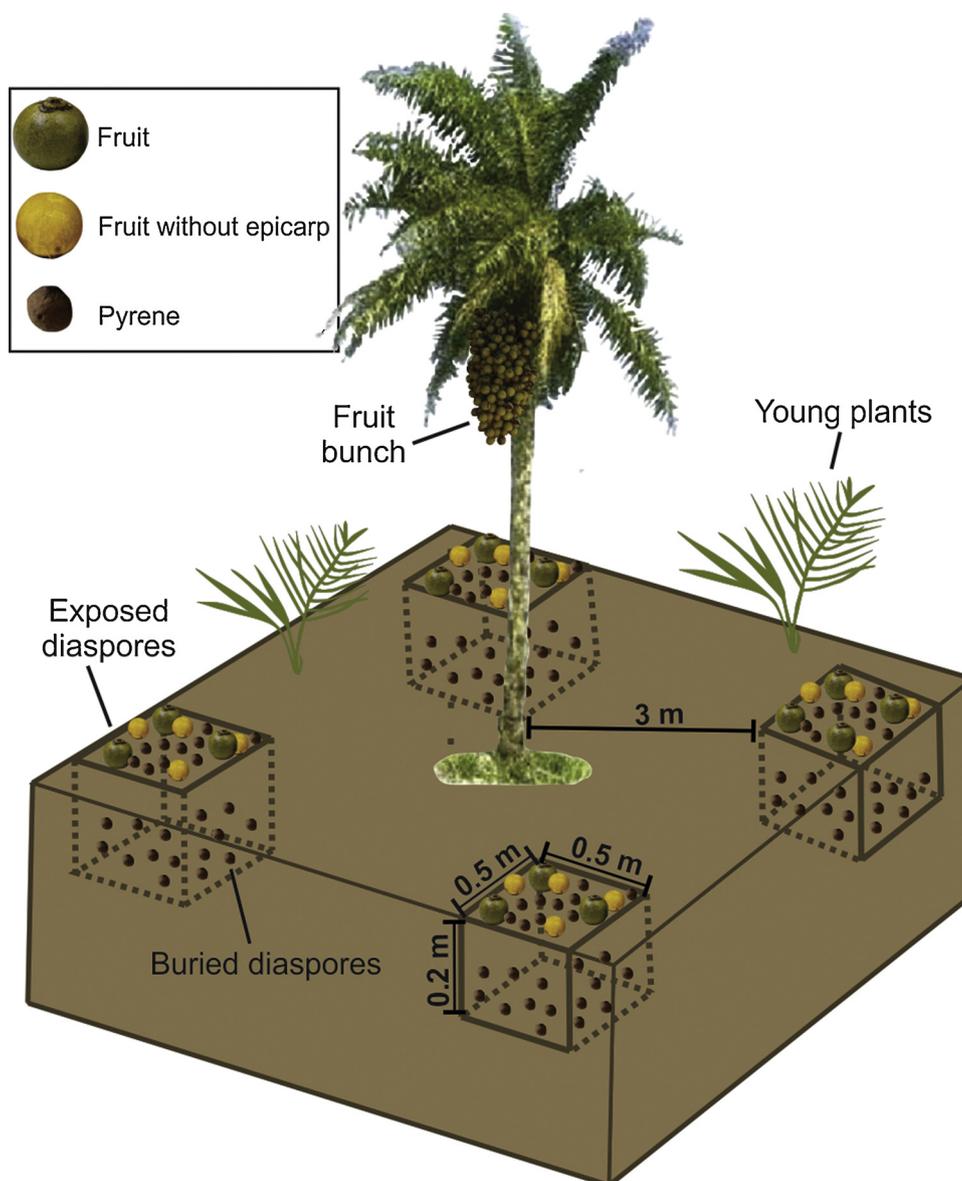
Fig. 2. Schematic illustrations of adult and young plants of *Acrocomia aculeata*, the types of diaspores, and sampling sites.

considered viable were capable of elongation; embryos considered vigorous had the capacity to grow into seedlings with leaf sheaths and roots (Ribeiro et al., 2011). Any embryos not showing development after cultivation for 30 days were tested for viability using tetrazolium (Ribeiro et al., 2010).

2.3. Soil characterizations

The soil samples were evaluated to determine their physical attributes: coarse sand (CS), fine sand (FS), coarse sand + fine sand (CSFS), silt (SILT), clay (CLAY); organic matter (OM), organic carbon (OC). The chemical attributes measured were: pH in water (pH), P-Mehlich (PM), residual P (PR), K, Ca, Mg, Al, H + Al (HAl). The data obtained from the chemical attributes were used to calculate: the sums of exchangeable bases (SEB), effective cation exchange capacity (t), percentage Al saturation (m), total cation exchange capacity (T), and base saturation percentage (V). The analyzes were performed at the Soil Analysis Laboratory of the Agricultural Sciences Institute of the Federal University of Minas Gerais - UFMG, Brazil, based on standard methodologies (Embrapa and Centro Nacional de Pesquisa de Solos, 2011).

2.4. Evaluations of soil seed bank dynamics indicators

The characteristics associated with the diaspores, seeds, and embryo productivity were considered SSB input indicators (Fig. 4). The total numbers of exposed and buried diaspores were determined in terms of the three post-dispersal developmental stages: intact fruits, fruits without the exocarp, and pyrenes (seeds surrounded by the endocarp) (Figs. 3a–c, 4). The total numbers of exposed and buried pyrenes were also recorded. The diaspores were opened using a manual bench vise, and the numbers of intact seeds in the exposed and buried diaspores were recorded. The embryos were excised from intact seeds with the aid of a scalpel, and the number of embryos obtained from exposed and buried diaspores were recorded.

The percentages of viable and vigorous intact embryos obtained in the previous evaluations were considered SSB maintenance indicators (Fig. 4). The viability and vigor of embryos from exposed and buried diaspores were determined by *in vitro* culturing, as previously described.

Characteristics associated with insect predation, microbial contamination, and/or physical damage to seeds were considered negative SSB output indicators (Fig. 4). The numbers of holes caused by
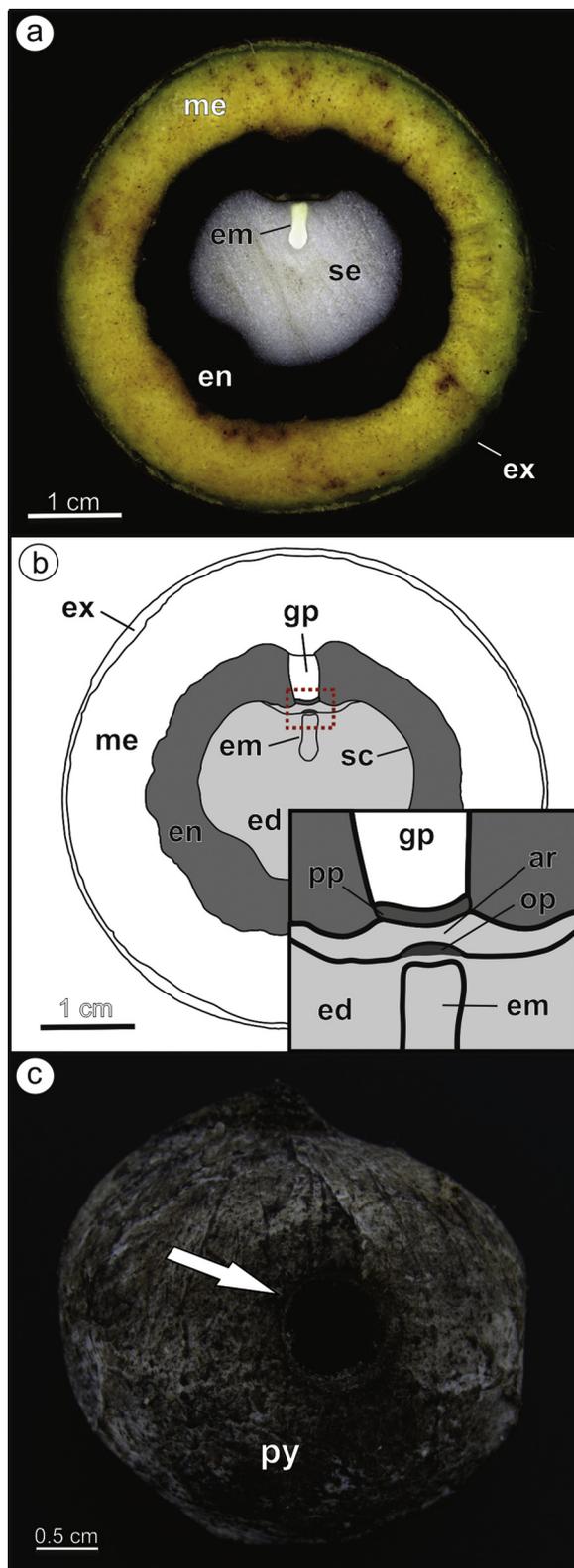
Fig. 3. Morphology of the diaspores of *Acrocomia aculeata*. (a) Longitudinal section of the fruit. (b) Schematic illustrations of fruit structures, with a detail of the micropylar region (dashed square in the lower right corner). (c) Pyrene (secondary dispersal unit) showing a hole caused by Bruchidae predation (Coleoptera: Chrysomelidae) (arrow). ar, Aryloid; ed, endosperm; em, embryo; en, endocarp; ex, exocarp; gp, germinative pore; me, mesocarp; op, operculum; pp, pore plate; py, pyrene; sc, seed coat; se, seed.

Bruchidae insect predation (Coleoptera: Chrysomelidae) in exposed and buried diaspores were recorded, based on the criteria established by Pereira et al. (2014) (Fig. 3c). The fruits were open (using a bench vise) and the numbers of exposed and buried diaspores that did not contain seeds were recorded. We also recorded: the numbers of partially consumed seeds obtained from exposed and buried diaspores; the numbers of seeds from exposed and buried diaspores showing necrosis typical of bacterial contamination (Ribeiro et al., 2011); the numbers of seeds obtained from exposed and buried diaspores showing fungal mycelium; and the numbers of seeds obtained from exposed and buried diaspores containing predator insects (Pereira et al., 2014).

Characteristics associated with the establishment of young plants were considered positive SSB output indicators (Fig. 4). Areas within a 10-meter radius around each adult plant were established (where the previously described collections were made) and the numbers of emergent young plants (individuals with no reproductive structures) and their heights were recorded.

2.5. Persistence and climatic control of soil seed bank dynamics

A. aculeata fruits were collected from 20 individuals in a natural population in the municipality of Montes Claros, Minas Gerais State, Brazil (S 16°42′34″ × W 43°52′48″) after their natural abscission to evaluate SSB persistence and climatic control of germination. The fruits were depulped mechanically and 3000 pyrenes (Fig. 3c) were sown (in a laterally open greenhouse) into furrows 8 cm deep in two different substrate types (washed sand or clayey soil) and maintained under daily irrigation, using tap water. Seedling emergence was evaluated on a weekly basis for five years. The maximum and minimum daily temperatures during the five-year evaluation period (and also of the historical series from 1961 to 2013) were obtained (from the National Institute of Meteorology – Inmet, for the municipality of Montes Claros) to calculate average temperatures and thermal amplitudes.

Five hundred pyrenes were removed from each substrate after the five year period to evaluate their viability and vigor in the simulated SSB. Those pyrenes were opened and the numbers of intact seeds recorded. The embryos were then excised from the seeds and submitted to *in vitro* culturing, as previously described.

In order to evaluate thermal control of germination, seeds were removed from the pyrenes (using a manual bench vise), disinfested in 6% sodium hypochlorite for 15 min, and treated with a commercial fungicide (Derosal Plus®) composed of carbendazim (150 g/L) and thiram (350 g/L), at 50% of its original concentration. The operculum, the structure that limits embryo expansion (Fig. 3b), was carefully removed from half of the seeds using a scalpel (Carvalho et al., 2015). The seeds (with or without the operculum) were then sown into polyethylene containers containing 20 g of sterilized vermiculite moistened with distilled water (at up to 80% retention capacity). Cultivation was carried out in germinators adjusted to 15, 20, 25, 30, 35 and 40 °C. The experiment was established using a randomized block design, with 12 treatments (seeds with or without operculum at six temperatures) and five replicates using 20 seeds each. After 30 days, all treatments were transferred to 30 °C (the temperature generally used for *A. aculeata* embryo culture and seed germination; Ribeiro et al., 2011, 2012b) and maintained at that temperature for another 30 days. Daily evaluations were then made, considering cotyledonary petiole protrusion as indicating germination (Ribeiro et al., 2011).

2.6. Statistical analyses

The adherence of the data on the freshly dispersed fruits and the characterization of the SSB (Fig. 4) to normal distributions were evaluated by means of the Kolmogorov-Smirnov test. When the absence of normality was verified, the Kruskal-Wallis nonparametric test was used. In the presence of normality, analysis of variance and the Tukey test were used for the data on the thermal control of germination. All of the
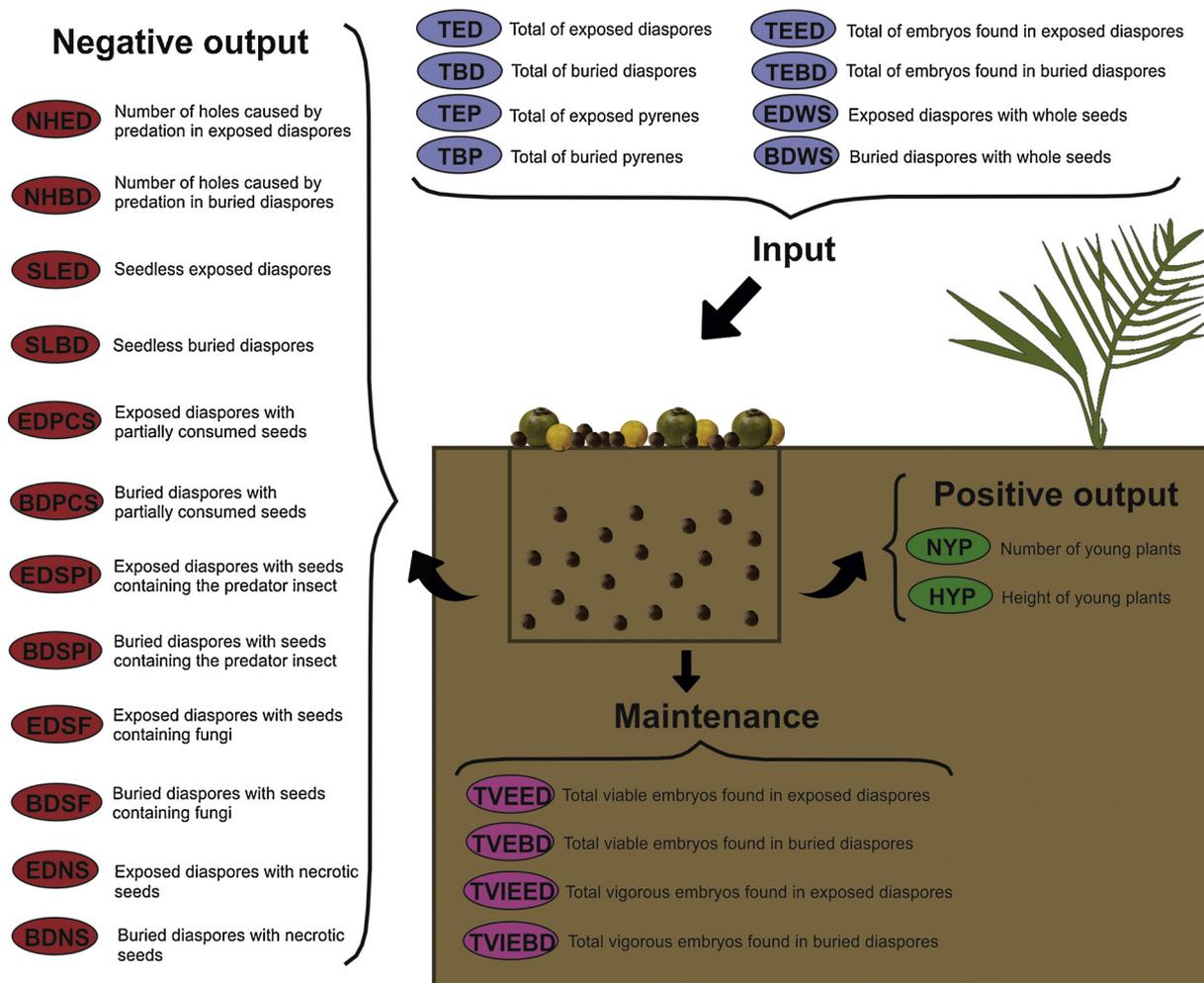
Fig. 4. Schematic illustration of the types of diaspores of *Acrocomia aculeata* and the criteria considered indicative of SSB input, negative output, maintenance, and positive output.

analyses were performed considering a 5% level of significance.

In the analysis of edaphic control of SSB dynamics, the diaspore characteristics evaluated were separated according to their meanings as indicators of SSB dynamics (input, maintenance or output) (Fig. 4). Soil characteristics and the SSB dynamics indicators were related to each other through principal components (PCA) multivariate analysis and correlation network analysis. The "princomp" function of R software was used in the study of principal components. The studies of correlation used graphical representations of the significant associations by the t-test, at a 5% level of significance, using the "corrplot" package in R software.

3. Results

3.1. Soil characterizations

Soil analysis data of the five areas studied are presented in Table 1. Areas 3 and 4, used for agricultural purposes, had higher fertility indicator values as compared with areas 1 and 2, which had been used as pasture. Area 5, despite being used only for pasture, showed fertility indicator values closest to areas 3 and 4. More clayey and less sandy soils have higher organic matter percentages, sums of bases, base saturations, and total and effective cation exchange capacity.

3.2. Evaluations of soil seed bank dynamics indicators

The embryo viability (mean value = 89%, $P = 0.089$) or vigor (mean value = 80%; $P = 0.056$) in freshly dispersed fruits did not vary between areas where SSBs were evaluated. There were, however, differences between individuals (mother plants) in terms of those same parameters within each area ($P < 0.0001$, for viability and vigor). Regarding the SSB evaluation, the areas and individuals differed in terms of the total numbers of diaspores, seeds, embryos, and embryo viability and vigor in exposed as well as buried diaspores ($P < 0.0001$, for all characteristics) (Fig. 5a–f). The highest means of total diaspores, seeds, embryos, and viable and vigorous embryos (obtained from exposed and buried diaspores) were found in area 3, while the lowest means were found in area 5 (except for the mean of total buried diaspores, which was lower in area 2, and vigorous embryos [obtained from exposed diaspores], which were higher in area 1).

The mean number of young plants was highest in area 4 and lowest in area 2 (Fig. 6a). There was a predominance of young plants with a height of less than 0.5 m and the highest means were found in area 5 (Fig. 6b).

3.3. Correlations between soil attributes and soil seed bank dynamics indicators

Multivariate analysis indicated characteristics with high correlations with the main components 1 and 2 (Fig. 7). Those characteristics,

Table 1
Physical-chemical characteristics of the soil in five areas with populations of *A. aculeata* in the Cerrado biome in central Brazil. Averages of 40 samples per area, followed by the standard error of the mean.

Characteristic	Area									
	1		2		3		4		5	
pH	5.56	± 0.06	5.41	± 0.05	6.18	± 0.06	5.83	± 0.17	5.67	± 0.09
PM (mg dm^{-3})	3.59	± 0.19	14.33	± 4.56	102.20	± 12.9	5.18	± 1.19	6.99	± 2.33
PR (mg dm^{-3})	48.03	± 0.52	48.33	± 0.63	41.67	± 0.39	41.30	± 0.60	46.25	± 0.59
K (mg dm^{-3})	191.10	± 8.53	183.50	± 6.77	333.60	± 44.1	430.33	± 11.2	409.9	± 16.2
Ca (cmolc dm^{-3})	2.93	± 0.21	3.65	± 0.21	6.35	± 0.17	8.72	± 0.34	5.13	± 0.23
Mg (cmolc dm^{-3})	1.21	± 0.05	2.26	± 0.73	2.03	± 0.08	4.71	± 2.89	1.68	± 0.08
Al (cmolc dm^{-3})	0.01	± 0.01	0		0		0		0.03	± 0.01
HAl (cmolc dm^{-3})	2.54	± 0.09	4.20	± 0.15	2.43	± 0.10	2.08	± 0.10	4.23	± 0.22
SEB (cmolc dm^{-3})	4.63	± 0.24	6.38	± 0.74	9.24	± 0.19	14.53	± 2.96	7.86	± 0.29
t (cmolc dm^{-3})	4.64	± 0.24	6.38	± 0.74	9.24	± 0.19	14.53	± 2.96	7.89	± 0.29
m (%)	0.33	± 0.16	0		0		0		0.45	± 0.21
T (cmolc dm^{-3})	7.16	± 0.27	10.54	± 0.74	11.67	± 0.18	16.62	± 2.94	12.09	± 0.35
V (%)	63.95	± 1.05	57.65	± 1.56	78.72	± 0.88	84.35	± 1.02	64.78	± 1.47
OM (dag Kg^{-1})	4.84	± 0.19	5.29	± 0.24	7.21	± 0.43	7.53	± 0.28	7.79	± 0.44
OC (dag Kg^{-1})	2.81	± 0.11	3.07	± 0.14	4.18	± 0.25	4.37	± 0.16	4.53	± 0.26
CS (dag Kg^{-1})	11.16	± 0.79	29.01	± 1.65	18.70	± 1.44	5.70	± 0.24	6.61	± 1.95
FS (dag Kg^{-1})	70.41	± 0.88	42.25	± 1.64	36.80	± 1.24	36.70	± 1.70	31.89	± 2.66
SILT (dag Kg^{-1})	8.35	± 0.45	13.00	± 0.90	20.13	± 1.18	29.75	± 1.28	36.03	± 1.26
CLAY (dag Kg^{-1})	9.45	± 0.33	15.75	± 0.63	24.38	± 0.79	27.85	± 1.17	25.63	± 0.72

pH – soil hydrogen potential; PM – phosphorus-Mehlich; PR – residual phosphorus; HAl – hydrogen + aluminum; SEB – sum of bases; t - effective cation exchange capacity; m – percentage of aluminum saturation; T – total cation exchange capacity; V – percentage of base saturation; OM – organic matter; OC – organic carbon; CS – coarse sand; FS – fine sand; Silt – silt; Clay – clay.

with correlation estimates > 0.7, were considered the most important in the multivariate study as they explained more than 50% of the variation of the main component.

Areas 3 and 4 showed more abundant SSBs, more robust inputs and maintenance indicators, and greater seedling establishment effectiveness (positive output) (Figs. 5–7). Area 3 presented a high estimate of the main component 1, and low scores of main component 2, which were related to the higher P-Mehlich estimates and some input (total diaspores, pyrenes, seeds, and embryos) and maintenance indicators (viable and vigorous embryos), as well as positive output indicators (numbers of young plants) (Fig. 7). In area 4, a low estimate of the main component 1 and a high value of main component 2 was evidenced. That area had higher titers for organic matter, K, Ca, SEB, t, T, clay, and silt, as well as fungal contamination and absence of seeds in the diaspores.

Area 5 had high estimates of principal components 1 and 2 (Fig. 7) and attributes associated with better soil quality (SEB, t, T, organic matter, percentage of K, Ca, clay) that were similar to area 4. Those two areas diverged, however, regarding the concentrations of Al and residual P (Table 1, Fig. 7). Area 5 also demonstrated high estimates for some characteristics associated with negative SSB outputs (fungal contamination, absence of seeds in the diaspores and, principally, predation) (Fig. 7).

Areas 1 and 2 had less abundant SSBs, and less robust input indicators that were associated with lower effectiveness in the establishment of young plants as compared with areas 3 and 4 (Figs. 5–7). Area 1 showed high estimates of the principal components 1 and 2, while Area 2 showed a high estimate of principal component 1 and a low estimate of component 2 (Fig. 7). Those areas showed higher estimates of residual P and characteristics associated with negative SSB outputs (predation), while area 1 had higher estimates of sand.

The analyses of correlation networks showed that the input, maintenance, and positive and negative SSB output indicators were not directly related to soil chemical attributes (except the concentrations of P and Al, and pH) (Fig. 8). The P-Mehlich was strongly related to input and maintenance indicators, although a negative correlation was observed between the input indicators and residual soil P.

The characteristics representing soil Al concentrations (Al, m, and HAl) were strongly correlated with some indicators of negative SSB outputs (predation and fungal contamination) (Fig. 8). There was an inversely proportional relationship between Al, m, and HAl in the analysis of main components, with positive SSB input, maintenance, and output indicators (Fig. 7). No significant negative correlations were found, however, between those characteristics in the correlation network analysis (Fig. 8). Characteristics related to greater soil water retention capacities (clay and organic matter) showed a strong positive correlation with some indicators of negative SSB outputs (necrosis and fungal contamination). Positive output indicators were not positively related to any of the soil characteristics evaluated.

3.4. Persistence and climate control of soil seed bank dynamics

In the greenhouse experiment conducted during a five year period, seedling emergence began in September-November every year (Fig. 9a). Emergence (mean of 6% per year considering both types of substrate) occurred in all years after periods with low thermal amplitudes and elevated average temperatures (Fig. 9a and b). That pattern was related to the seasonality of the study region, as confirmed by historical climate data, with the greatest thermal amplitudes occurring during dry winters and temperature elevations in the spring, preceding the rainy season (Figs. 9b, 10). Seedling emergence after the second year from diaspores sown into sand was greater than that of clayey soils. After the five-year evaluation period 40% seedling emergence in the sand and 22% in the soil were found. Thirteen percent of total sown pyrenes had intact seeds; 6% had intact, 4.8% had viable, and 2.1% had vigorous embryos.

In the experiments conducted under laboratory conditions, the highest germination percentages were observed among seeds without their operculum, and at temperatures between 20 and 40 °C; there were no significant differences between the effect of the temperatures in that range (Fig. 11). The lowest germination percentages were observed at 15 °C. Seeds with their operculum intact showed no germination during the time they were held at different temperatures. After transfer to a constant 30 °C temperature, seeds without their operculum and initially held at 15 and 20 °C showed increased germination; likewise, seeds with their operculum intact and previously held at 40 °C showed significant increases in their germination percentages after transfer to 30 °C.
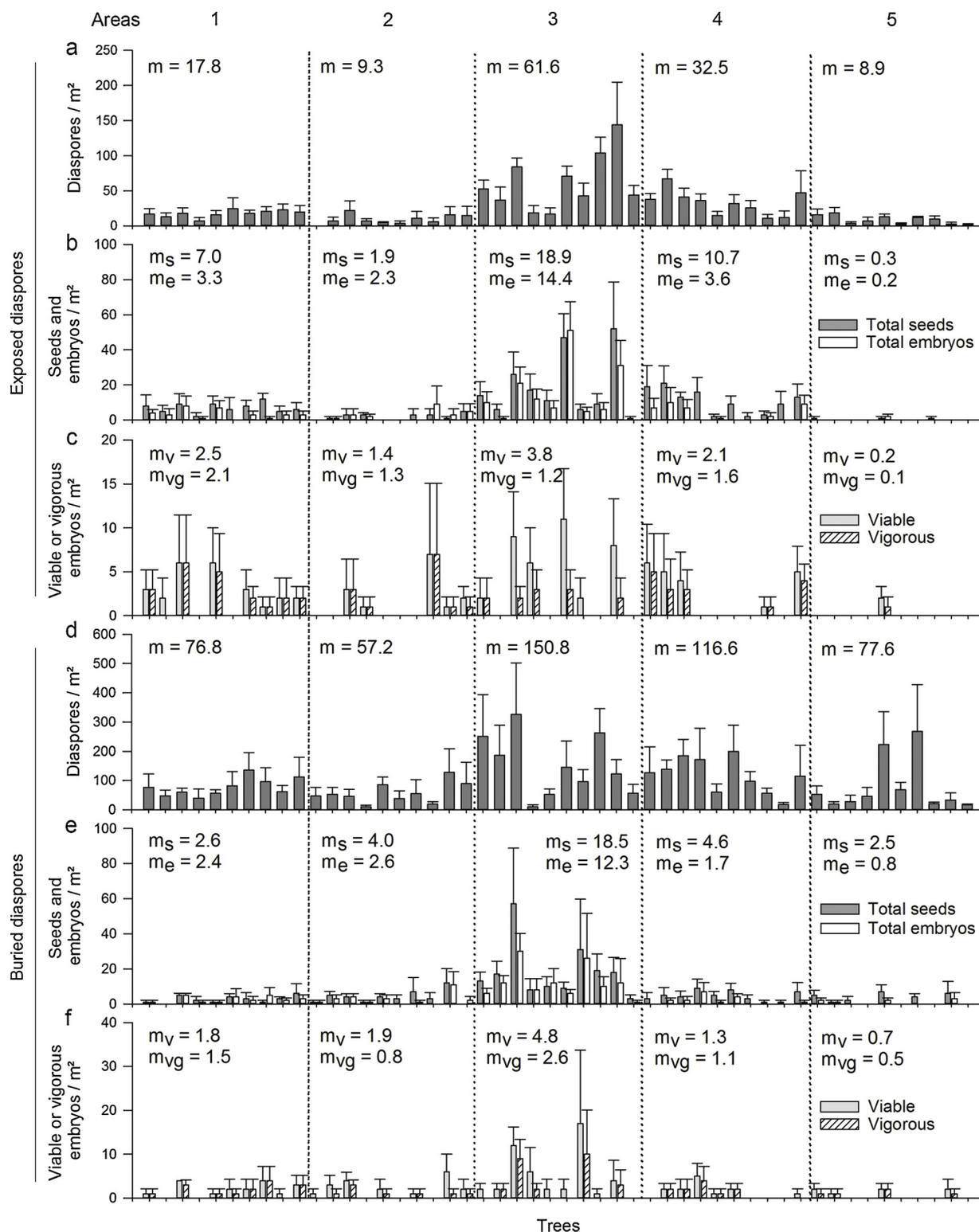
Fig. 5. Characterizations of the SSBs of five populations of *Acrocomia aculeata* in the Cerrado biome in central Brazil. (a, d) Numbers of diaspores, (b, e) seeds, and embryos, (c, f) viable and vigorous embryos produced by exposed and buried diaspores. m, Mean; m_s, mean of seeds; m_e, mean of embryos; m_v, mean of viable embryos; m_{vg}, mean of vigorous embryos. The vertical bars indicate the standard errors of the means.

4. Discussion

The present work represents the first detailed study relating soil attributes and climatic conditions to SSB dynamics in neotropical environments. Our results showed that edaphic factors influenced mainly the incorporation of viable seeds (inputs) into macaúba palm SSB, but also affected the death of the embryos previously included in SSB (negative outputs), while climatic factors controlled conversion of embryos to seedlings (positive outputs). Soil seed banks maintenance is important for the reproductive success of *A. aculeta* and may be useful for agricultural management.
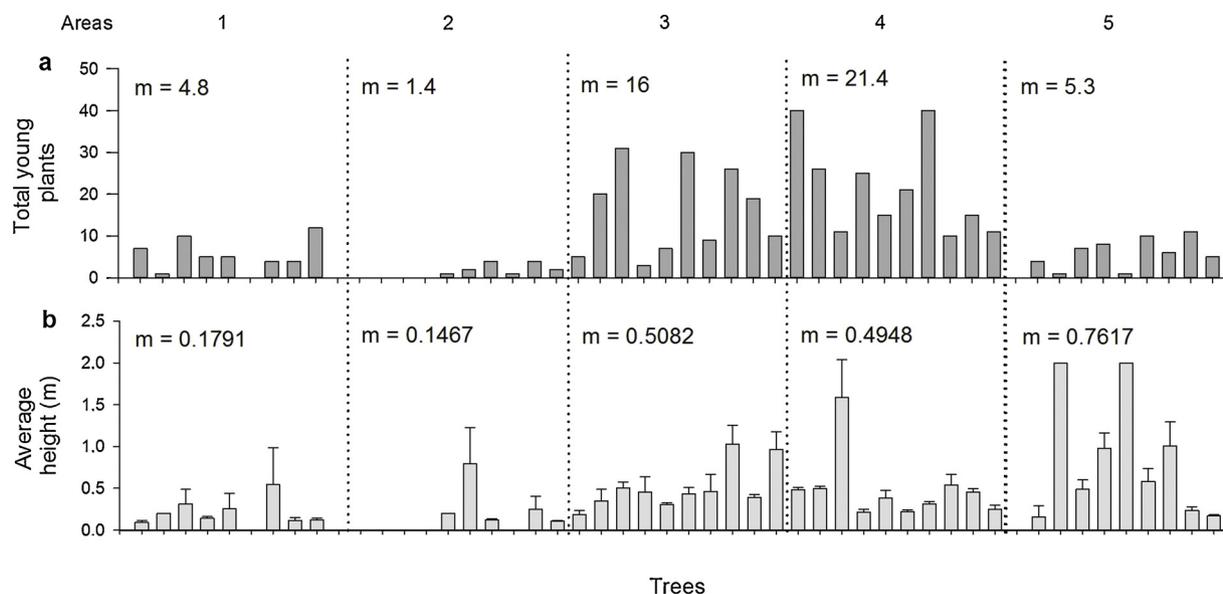
Fig. 6. Characterizations of young plants of five populations of *Acrocomia aculeata* in the Cerrado biome in central Brazil. (a) Numbers and (b) average heights of young plants. m, Mean. The vertical bars indicate the standard errors of the means.

4.1. Soil seed bank classification

Macaúba palms form persistent SSBs, which, according to Thompson et al. (1998) and Csontos and Tamás (2003), represent the fraction of the seed population that, after dispersal, germinates or dies within a period of more than one year. Our results showed that SSBs had a mean of two viable embryos and one vigorous embryo per m² (Fig. 5). Additionally, the greenhouse experiments showed that at the end of the five-year evaluation period, approximately 5% of the embryos still remained viable. Several palm species from seasonal environments produce long-lived seeds, and commonly form persistent SSBs (Orozco-Segovia et al., 2003; Ribeiro et al., 2011; Long et al., 2014). Evidence from the literature, combined with our results, allowed us to identify the main factors associated with the persistence mechanisms of *A. aculeata* seeds in the soil: structural protection of the embryo, orthodoxy, and resistance to germination.

Dispersed macaúba seeds are protected by pericarp structures under natural conditions (Mazzottini-dos-Santos et al., 2015; Rodrigues-Junior et al., 2016) (Fig. 3a and b). After consumption of the mesocarp by disperser animals (normally bovines and rodents), the secondary dispersal unit is the pyrene – which protects the embryos against high temperatures and predation (Ribeiro et al., 2011; Mazzottini-dos-Santos et al., 2015; Rodrigues-Junior et al., 2016) (Fig. 3c). The seeds have a phenolic-laden tegument and an endosperm composed of cells whose walls are rich in mannans that impart resistance to those tissues (Mazzottini-dos-Santos et al., 2016). Additionally, the embryo is protected by the germinative pore plate (formed by the sclerification of the locular epidermis), aryloids, and the operculum (formed by the opercular seed coat and micropylar endosperm) (Ribeiro et al., 2011; Mazzottini-dos-Santos et al., 2015; Carvalho et al., 2015). Together, those structures contribute to the persistence of the SSB until the appearance of favorable conditions for seedling establishment.

Orthodox seeds are tolerant of desiccation and low temperatures (Hong and Ellis, 1996). Ribeiro et al. (2012a) showed that macauba seeds are tolerant of desiccation (water contents below 5%) and freezing, and can remained viable for 12 months when the fruits are kept exposed to environmental conditions (direct sunshine and rains) on the soil. Macaúba seeds are oleaginous and therefore prone to deterioration (Barreto et al., 2014; Ribeiro et al., 2015), although studies have shown that the species has efficient physiological mechanisms (involving compounds such as abscisic acid and vitamin E) that help

prevent lipid peroxidation and fungal infections (Barreto et al., 2014; Ribeiro et al., 2015).

For seeds to persist in a SSB they must remain alive, and not germinate (Long et al., 2011; 2014). Dormancy is the blockage of seed germination caused by intrinsic mechanisms, and has adaptive value (Baskin and Baskin, 2014). *A. aculeata* seeds demonstrate pronounced dormancy related to the low growth potential of the embryo (which cannot initially overcome the resistance of the operculum and pore plate) (Ribeiro et al., 2011, 2013; Mazzottini-dos-Santos et al., 2018) (Fig. 3b). Overcoming dormancy in that species occurs gradually and is associated with alterations of the gibberellin/abscisic acid balance in the embryo that promotes its growth (Bicalho et al., 2015; Ribeiro et al., 2015). That hormonal balance is usually altered after the perception of environmental signals indicating favorable conditions for seedling establishment (Lee et al., 2010; Yan et al., 2014; Mazzottini-dos-Santos et al., 2018).

4.2. Influence of edaphic factors on soil seed bank dynamics

The maintenance of intact *A. aculeata* embryos in the SSB is little affected by edaphic factors, although elevated levels of P, Al, organic matter, clay, and sand influence SSB inputs and negative outputs (Fig. 12). The indicators associated with SSB input factors were positively influenced by matrix plant productivity and P-Mehlich concentrations. The high productivity of macaúba palms (each adult plant can produce more than 4000 fruits annually; Pires et al., 2013) provides large numbers of diaspores that are not all able to successfully germinate (due to dormancy) and can thus compose the SSB. Several studies have demonstrated the importance of P as a key element for increasing plant productivity in tropical regions (Balemi and Negisho, 2012; Tawaraya et al., 2012). In the present study, the areas with the best input and positive output indices for SSB factors had more fertile soils (Figs. 5 and 6). Those results corroborated the work of Ratter et al. (1996) and Motta et al. (2002), who noted that macaúba palm stands are indicative of eutrophic soils.

Elevated levels of Al, sand, clay, and organic matter were strongly correlated with negative SSB outputs. The main factors causing seed mortality in SSB are usually: aging, predation, and bacterial and/or fungal infections (Wagner and Mitschunas, 2008). Post-dispersal seed predation commonly results in reductions of host plant reproductive potentials (Janzen, 1971; Delobel et al., 1995; Kolb et al., 2007; Pereira
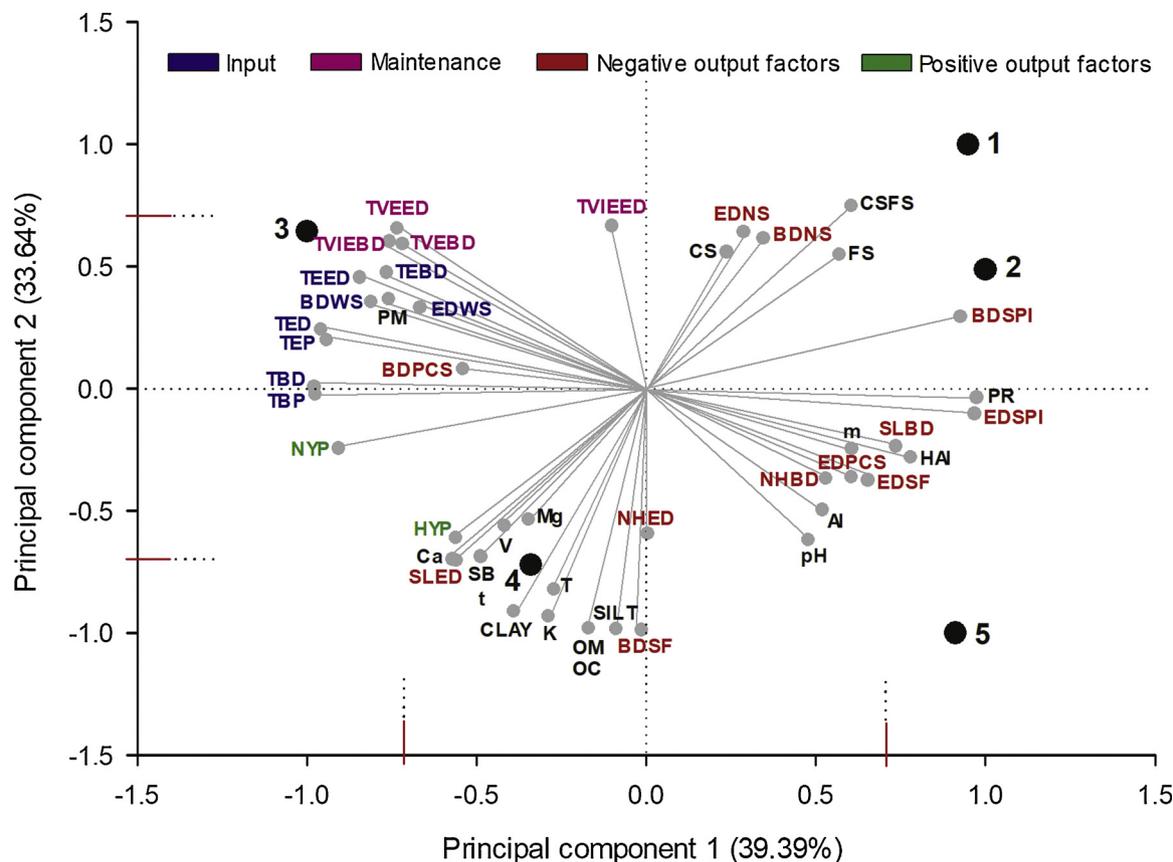
Fig. 7. Graphical dispersion of the main components of the variables related to the study areas (black circles, numbered 1–5), soil attributes and characteristics of the diaspores and young plants of *Acrocomia aculeata*, considered indicative of SSB input, maintenance, negative output, and positive output soil attributes: hydrogen potential (pH); P-Mehlich (PM); residual P (PR); K; Ca; Mg; Al; H + Al (HAl); sum of bases (SEB); effective cation exchange capacity (t); percentage aluminum saturation (m); total cation exchange capacity (T); percentage base saturation (V); organic matter (OM); organic carbon (OC); coarse sand (CS); fine sand (FS); coarse sand + fine sand (CSFS); silt (SILT); clay (CLAY). Characteristics of the diaspores: total of exposed diaspores (TED); total of exposed pyrenes (TEP); number of holes caused by predation in exposed diaspores (NHED); seedless exposed diaspores (SLED); exposed diaspores with whole seeds (EDWS); exposed diaspores with partially consumed seeds (EDPCS); exposed diaspores with seeds containing the predator insect (EDSPI); exposed diaspores with seeds showing fungal growth (EDSF); exposed diaspores with necrotic seeds (EDNS); total of buried diaspores (TBD); total of buried pyrenes (TBP); number of holes caused by predation in buried diaspores (NHBD); seedless buried diaspores (SLBD); buried diaspores with whole seeds (BDWS); buried diaspores with partially consumed seeds (BDPCS); buried diaspores with seeds containing the predator insect (BDSPI); buried diaspores with seeds showing fungal growth (BDSF); buried diaspores with necrotic seeds (BDNS); total of embryos found in exposed diaspores (TEED); total viable embryos found in exposed diaspores (TVEED); total vigorous embryos found in exposed diaspores (TVIEED); total of embryos found in buried diaspores (TEBD); total viable embryos found in buried diaspores (TVEBD); total vigorous embryos found in buried diaspores (TVIEBD); number of young plants (NYP); height of young plants (HYP).

et al., 2014), often with severe impacts on population dynamics (Janzen, 1971; Ramírez and Traveset, 2010). As shown here, insect predators of *A. aculeata* seeds prefer clayey soils, corroborating the observations of Ribeiro et al. (2011). Our results demonstrated that high Al concentrations in the soil also increased the probability of insect attacks (Fig. 8). It is interesting that high clay contents are generally associated with the most fertile soils, while the opposite occurs with Al-rich soils (Embrapa and Centro Nacional de Pesquisa de Solos, 2011). It is therefore possible to conclude that macaúba seed predation is favored by extreme (high or low) soil fertility conditions.

Pathogen contamination exerts an important influence on soil seed dynamics (Chee-Sanford et al., 2006; Wagner and Mitschunas, 2008). Fungal colonization rates of seeds in the soil are normally influenced by soil moisture (Schafer and Kotanen, 2003; Mordecai, 2012), soil type (Wagner and Mitschunas, 2008), fungal density (Horn, 2006), and by fruit characteristics (Janzen, 1977). Organic matter influences fungal attack rates on seeds (Wagner and Mitschunas, 2008), as it increases soil moisture and serves as a food source for saprophytic microorganisms (Pitty et al., 1987; Schafer and Kotanen, 2003; Wagner and Mitschunas, 2008). Our results showed that macaúba seeds were more susceptible to necrosis in sandy soils – the first report of that result in a

tropical region (Fig. 8). However, interference from other factors (not evaluated) cannot be ruled out (such as flooding – a common occurrence in sandy alluvial soils).

While soil characteristics strongly influenced the persistence of SSB seeds the maintenance indicators of intact macauba embryos in the SSB (embryo viability and vigor) were independent of soil characteristics – a feature that would favor the maintenance of its SSB in a wide range of environments (Long et al., 2009, 2014).

4.3. Roles of climatic factors in positive soil seed bank outputs

Climatic factors influence germination control and the gradual activation of mechanisms leading to overcoming dormancy in macaúba seeds, and thus modulate positive SSB outputs. That response reflects sophisticated mechanisms that favor germination only under conditions amenable to seedling establishment. The seeds of most species demonstrating dormancy will experience a series of temperature variations and soaking cycles, until changes occur in their ability to germinate (Baskin and Baskin, 2014). Our results showed that high temperatures are required for overcoming dormancy, and that seedling emergence occurs after periods of low thermal amplitude (winter) and
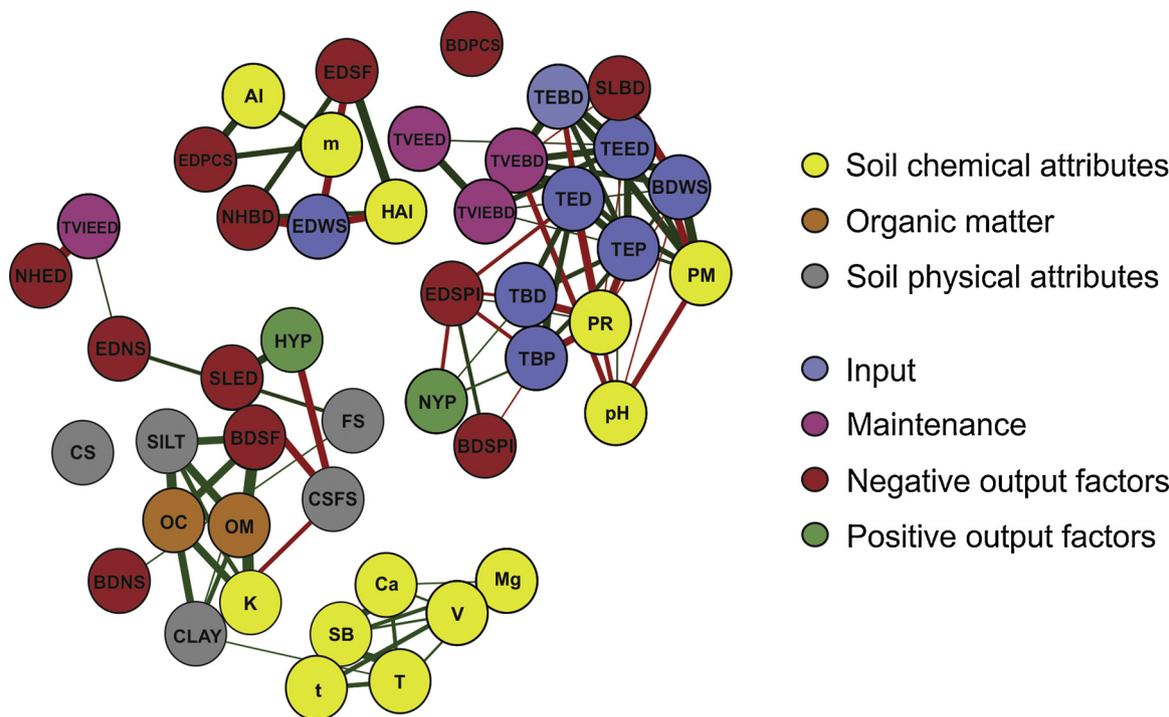
Fig. 8. Estimated correlation network based on soil attributes and the characteristics of the diaspores and young plants of *Acrocomia aculeata* related to SSB input, maintenance, positive output, and negative output. Red and green lines represent, respectively, negative and positive significant Pearson correlations by and *t*-test (P ≤ 0.05). Line widths are proportional to the correlation estimates. Soil attributes: soil hydrogen potential (pH); P-Mehlich (PM); residual P (PR); K; Ca; Mg; Al; H + Al (HAl); sum of bases (SEB); effective cation exchange capacity (t); percentage aluminum saturation (m); total cation exchange capacity (T); percentage base saturation (V); organic matter (OM); organic carbon (OC); coarse sand (CS); fine sand (FS); coarse sand + fine sand (CSFS); silt (SILT); clay (CLAY). Characteristics of the diaspores: total of exposed diaspores (TED); total of exposed pyrenes (TEP); number of holes caused by predation in exposed diaspores (NHED); seedless exposed diaspores (SLED); exposed diaspores with whole seeds (EDWS); exposed diaspores with partially consumed seeds (EDPCS); exposed diaspores with seeds containing the predator insect (EDSPI); exposed diaspores with seeds showing fungal contamination (EDSF); exposed diaspores with necrotic seeds (EDNS); total of buried diaspores (TBD); total of buried pyrenes (TBP); number of holes caused by predation in buried diaspores (NHBD); seedless buried diaspores (SLBD); buried diaspores with whole seeds (BDWS); buried diaspores with partially consumed seeds (BDPCS); buried diaspores with seeds containing the predator insect (BDSPI); buried diaspores with seeds showing fungal contamination (BDSF); buried diaspores with necrotic seeds (BDNS); total of embryos found in exposed diaspores (TEED); total viable embryos found in exposed diaspores (TVEED); total vigorous embryos found in exposed diaspores (TVIEED); total of embryos found in buried diaspores (TEBD); total viable embryos found in buried diaspores (TVEBD); total vigorous embryos found in buried diaspores (TVIEBD); number of young plants (NYP); height of young plants (HYP).
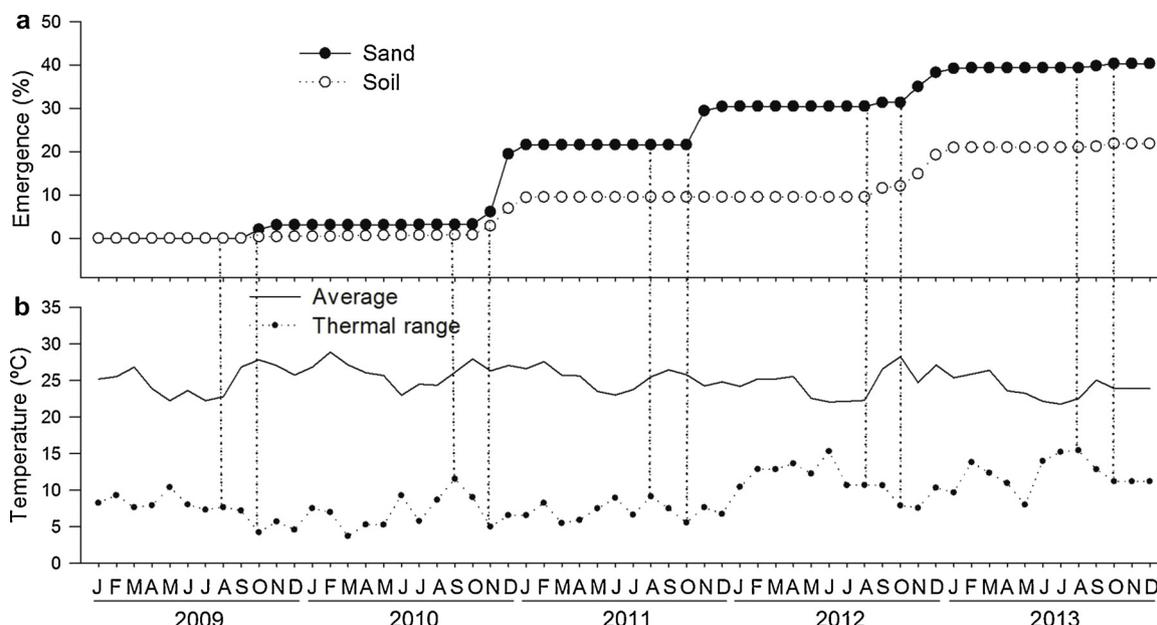
Fig. 9. Emergence of *Acrocomia aculeata* seedlings from simulated seed banks in two substrates (sand and clayey soils) under greenhouse conditions during five years (a). Mean temperatures and thermal ranges during the evaluation period (b).
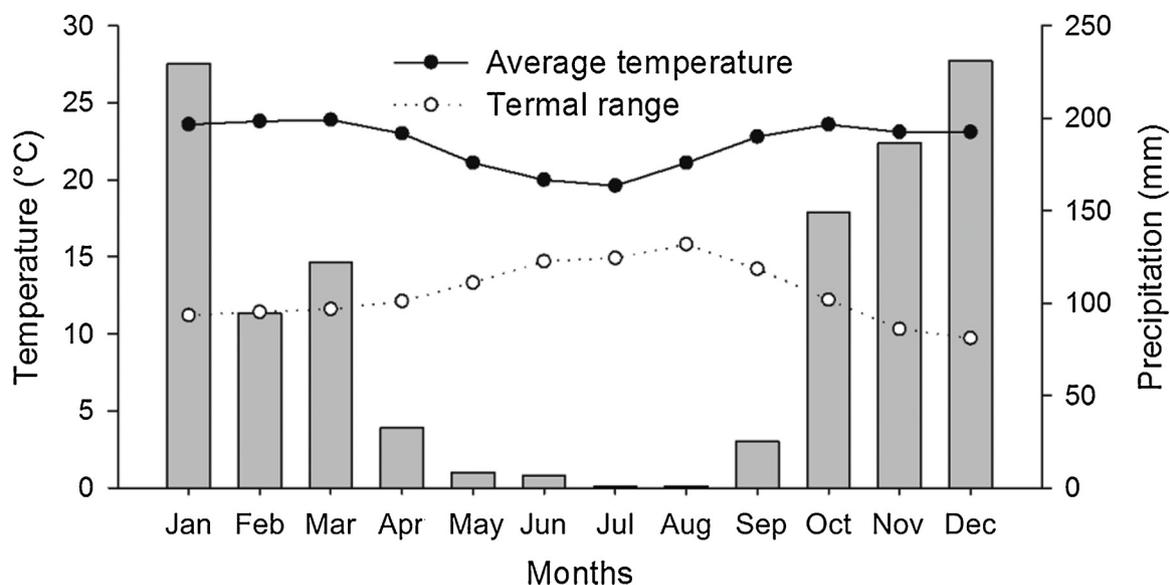
Fig. 10. Historical series (1961–2013) of average temperatures, thermal ranges, and monthly precipitation for the municipality of Montes Claros, northern Minas Gerais State, Brazil.

temperature increases (early spring) that signal the beginning of the rainy season (Figs. 9, 11). These findings lead us to conclude that the macaúba SSB, in addition to representing a reserve of genetic material, constitutes an important part of the plant's life cycle, contributing to adjustments of its development to environmental conditions in a general pattern previously observed in several species (Benech-Arnold et al., 2000; Ooi, 2012; Pakeman et al., 2012; Bewley et al., 2013; Long et al., 2014).

4.4. The role of the soil seed bank in A. aculeata reproduction and its adaptation to environmental events

Soil seed banks favor the conservation and maintenance of natural

populations of macaúba palms through time and space. *A. aculeata* is a pioneer species, widely distributed in the tropical Americas, and present in five major Brazilian biomes (Amazon, Cerrado, Caatinga, Atlantic Forest, and Pantanal) (Lorenzi, 2006; Lorenzi et al., 2010; Colombo et al., 2017) (Fig. 1) – and usually associated with anthropogenically modified environments (Motta et al., 2002; Lorenzi, 2006; Colombo et al., 2017). Although eutrophic soils show better results in terms of SSB dynamics, the maintenance of intact embryos in the soil independent of edaphic factors favors the reproductive success of the species and its wide distribution (Fig. 12). Climatic conditions can modulate SSB seed outputs, allowing the synchronization of seed germination with conditions favorable to seedling establishment – a response known to be important to the success of various species
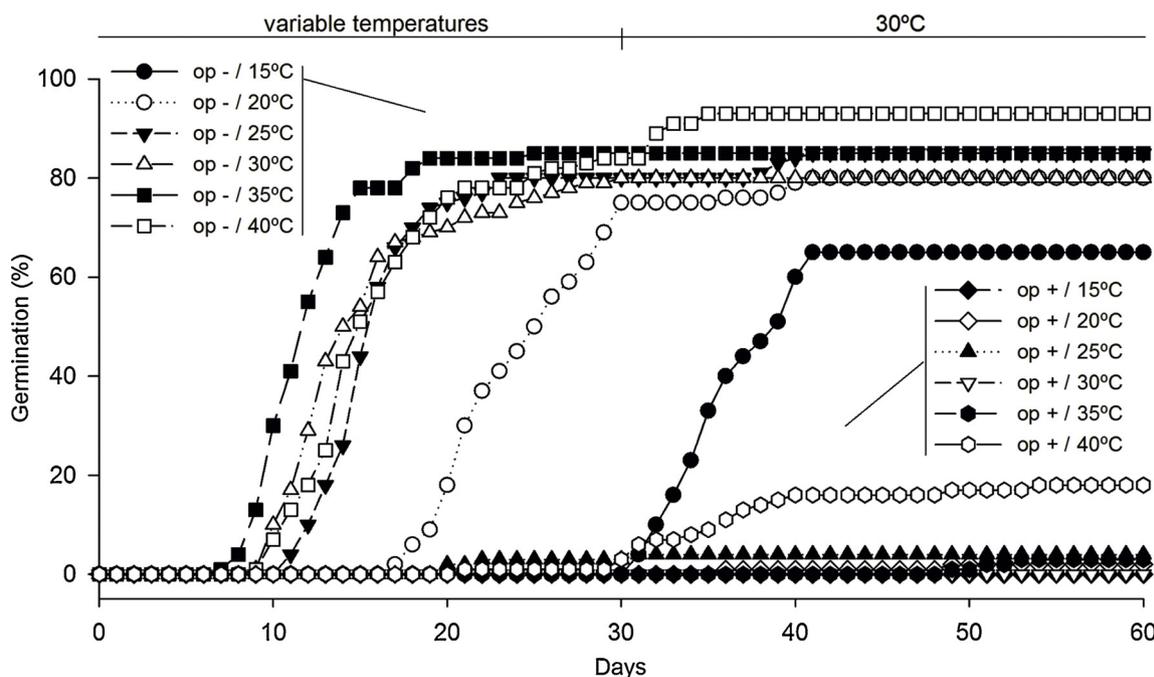

Fig. 11. Percentage of germination of *Acrocomia aculeata* seeds with intact operculum (op +) or without operculum (op-) sown onto vermiculite and submitted to different temperature, regimes during 30 days, and subsequently to a constant temperature of 30 °C for 30 days. Treatments followed by the same vertical line on the right do not differ statistically, by the Tukey test, at a 5% level of significance.
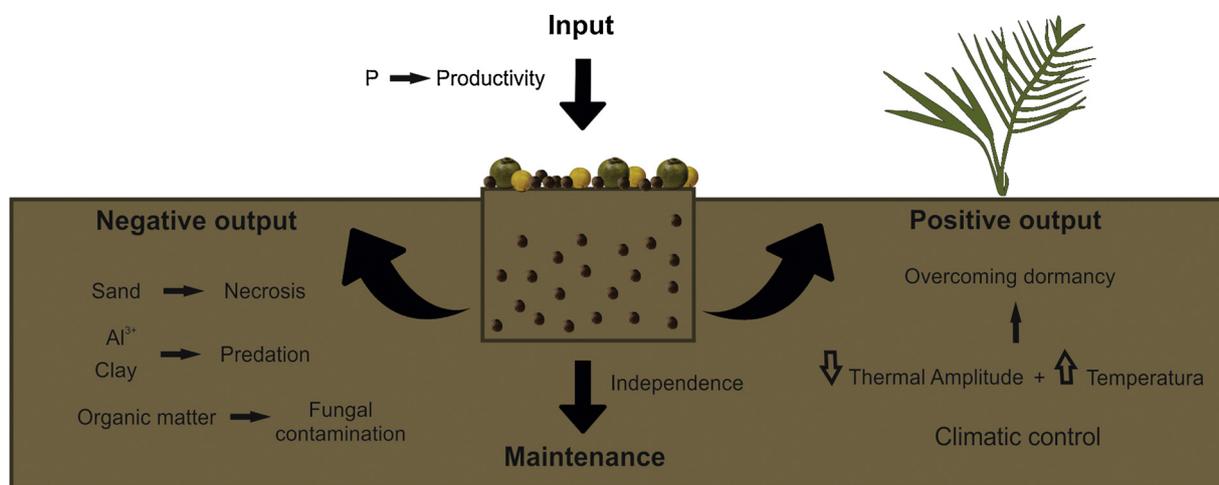
Fig. 12. Schematic illustration of the influences of edaphic and climatic factors on the dynamics of the SSBs of *Acrocomia aculeata*.

(Thompson and Grime, 1979; Pakeman et al., 2012; Long et al., 2014; Oliveira et al., 2017).

Due to the high potential for agro-industrial uses of *A. aculeata*, especially for biofuel production, several initiatives have been directed at its domestication, including efforts of enrichment and the management of natural populations (Pires et al., 2013; Cardoso et al., 2017; Colombo et al., 2017). In that context, our results indicate that: i) the abundance and persistence of a SSB signals the possibility of enriching natural populations through the management of naturally produced seedlings; ii) pyrene persistence in the soil opens the perspective of direct sowing (after treatment to overcome dormancy) that would reduce costs related to seedling planting and acclimatization; and, iii) the management of soil P and Al concentrations has the potential to favor reproduction and the population dynamics of the species.

5. Conclusions

Macaúba palm trees form persistent soil SSBs. Phosphorus is the main edaphic factor associated with SSB input, while high concentrations of Al and clay (due to insect predation), organic matter (fungal contamination), and sand (microbial contamination) contribute to negative SSB outputs. The maintenance of intact embryos, however, was found to be independent of the edaphic factors examined. The association between high thermal amplitudes in the winter and high temperatures in the spring promotes overcoming dormancy, and favors seedling establishment in the rainy season. The high productivity of adult plants, the ability to maintain viable embryos in the soil, and seed dormancy all contribute to the control of SSB dynamics, favoring the reproductive success of *A. aculeata* and its wide geographic distribution.

Declaration of Competing Interest

None.

Acknowledgements

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