Is the Assisted Enrichment efficient to the recovery of the Tropical Semi-deciduous Forests?

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Research Article

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Abstract

Due to their high natural fertility, Tropical Semi-deciduous Forests have been significantly impacted in recent decades, becoming fragile and requiring interventions for their restructuring. Among the methodologies, assisted enrichment has stood out for the recovery of already forested environments, with the introduction of species that promote the successional development of these environments. In this context, we evaluated the initial establishment of different species using direct seeding and seedling planting techniques as enhancers for Subdeciduous Tropical Forests. We used *Astronium urundeuva*, *Eugenia uniflora*, and *Hymenaea courbaril* as enriching species. The characterization of the influence of environmental characteristics was based on biotic and abiotic data from the sampled areas. In the study, we observed greater initial establishment of individuals under seedling planting, with higher survival compared to direct seeding. Even with low emergence, direct seeding showed higher relative growth compared to seedling planting. *A. urundeuva* and *E. uniflora* showed higher estimated survival in seedling planting and direct seeding, respectively, while *H. courbaril* showed similar establishment in both techniques. No significant influence of environmental parameters on the establishment of individuals was observed. Despite the low emergence of individuals, we do not dismiss direct seeding as an enhancer, considering its cost-benefit ratio.

Introduction

Due to the natural high fertility of their grounds, Tropical Semi-deciduous Forests (TSF) have been intensively exploited in recent decades (Durigan et al. 2000; IBGE 2012), remaining preserved in South America only 10% of this environment (Pio et al. 2023). Exacerbated by the intensity of the anthropic actions and ineffectiveness in public politics, this climate that once was home to a rich biodiversity, now is at risk and degraded (Law 2014).

Their fragility demands actions such as techniques of habitat restoration techniques and community reestablishment to ensure vegetation conservation and recovery (IMASUL 2016). However, what is observed in these regions after implantation is a stagnation of the successional development, stopping, at least for now, the establishment of new species (Lorenzoni-Paschoa et al. 2019).

To mitigate these effects, assisted enrichment presents itself as a promising technique to grant the structural reestablishment of habitats (Perumal et al. 2017). Consisting in the insertion of species in an ecosystem, assisted enrichment aims to promote the natural development of different individuals in an area, enabling an easy range of new successional stages (Rapport & Montaginini 2014).

In assisted enrichment, as in other restoration techniques, the purpose scope of the objectives is given by the relations of the selected species (Sampaio et al. 2021). Option to facilitator species that have a positive interactions that enable the settlement of others specimens to the flora (Fedriani et al. 2019), and generalists' plants, for their broad distribution and easy acclimation to the ambience (Silva 2024), representes essential characteristics to assisted enrichment's success.

Selecting the most suitable technique for the environment also benefits assisted enrichment (Sampaio et al. 2015). Both direct seeding and planting seedlings have positive results as enrichers (Atondo-Bueno et al. 2018; Wasli et al. 2014), but comparative gap persist regarding their efficiency in their initial establishments, especially in TSF, leaving the most recommended technique for each environment uncertain (Marshall 2021).

In the state of Mato Grosso do Sul, due to the fragility and scarcity of remnants of TSF (Damaceno-Junior et al. 2018), the selection of species, as well as techniques that are best suited to secondary vegetation formations are major challenges when aiming to integrate plant components with the other components of the ecosystem, in order to restore functional attributes and promote succession in these areas (Almeida 2016).

In view of these aspects, the goal of this study was to assess the efficiency in the establishment of different species under the direct sowing and planting of seedlings as enrichment as TSF in Mato Grosso do Sul, evaluating the influence of environmental parameters under techniques and species with superior initial performance superior initial performance.

Methods

Study area

The study happened in the region of Southern Center of Mato Grosso do Sul, Brazil (Fig. 1), in the areas of Municipal Natural Park of Gloria de Dourados (PNGMD), in Gloria de Dourados (54°12'56,00"S e 22°24'15,00"O) and The Experimental in Agricultural Sciences' Farm (FAECA) in Federal University of Grande Dourados, in Dourados (55° 59'55,00"S e 22°14'24,00"O)

[Figure near here]

The Studied areas characterize themselves as TSF. The grounds have characteristic of Dystrophic Red Latosol (EMBRAPA 2006) which, basing in the Solo Texturing's Class (Lemos & Santos 1996), is observed that FAECA has a very clayey ground and PNMGD is observed to have an Open Sand ground (Arai et al. 2010). The areas also count with mild annual precipitation of 1.410 mm, counting with the rainy period between the months of november and january and between the low temperature's months of july and august (Fietz & Fisch 2008). Rainfall and temperature data for the areas during the development of the study are available in Figure S1.

Studied species

It was selected three regional native species, the *Astronium urundeuva* (M. Allemão) Engl, *Eugenia uniflora* L. and *Hymenaea courbaril* L. (Table S1).

For the sowing, seeds were selected in 2021's fructification, occurring near the studied area. The collected seeds were packaged in impermeable plastics' bags and kept in a cold room (6–9 ºC and 60–65% in relative humidity), remaining until the experiment was set up (between 10 and 15 days of storage. To evaluate the viability of the seeds, it was applied Tetrazólio's Test (Silva & Aguiar 1998) with base in 100 shown individuals, gaining a viability of 86 percent to *A. urundeuva*, 92 percent to *E. uniflora* e 100 percent to *H. courbaril*.

The seedlings were obtained in publics' vivarium (Municipal Vivarium of Dourados, Municipal Vivarium of Gloria de Dourados and Experimental Vivarium of FAECA) and particulars' vivarium. The medium size of seedlings of *H. courbaril* wes 25 cm, *E. uniflora* 15 cm and *A. urundeuva* 20 cm.

Sampling design

The study occurred in november of 2021 and had the observing period of 12 months. The disposition of direct planting of seedling and sowing in the interior, as demonstrated in Fig. 2. Both techniques were implemented entering 20m of fragment with the intent to avoid the influence of the affects on the edge of data.

[Figure near here]

Seedling's plantation

Ten 12x14m plots were planted in each sampling area, with a minimum spacing of 20 meters between plots. Thirty individuals were planted in each plot, ten of each species. They were planted in 20 cm holes in 5 transects, spaced 2x2 meters apart. Each transect had 6 individuals planted interspersed, respecting the presence of two individuals of the same species per transect.

Direct sowing

Ten nurseries were set up in each sampling area, each with a minimum distance of 20 m between the nurseries. Each nursery had four 1x1m cribs, 5 cm deep. Ten seeds of a single species were sown in each crib, with the exception of the Mix crib, where ten seeds of each species were sown together. A control nursery was also placed in each sampling area in order to assess the natural occurrence of the species studied.

Monitoring the development

For planting seedlings, observations were made at 0 (measurement at the time the study was set up), 90, 180, 270 and 360 days after planting. For direct sowing, observations were made at 90, 180, 270 and 360 days after sowing. The emergent individuals in each cradle were labeled and their development monitored individually.

Collecting data

To quantify the quality of the studied forest habitat, data were collected on vegetation, environmental variables, soil, and litter

For the vegetation variables, a phytosociological survey was carried out in both the plots and the nurseries of all the trees with at least a 5 cm DBH (diameter at breast height). The species were identified by consulting specialists and the DDMS herbarium. All the usual phytosociological parameters were described using the FITOPAC 2 program (Shepherd 2010), which can be found in Table S2.

nEvironmental variables measured included temperature and relative humidity (using a thermohygrometer), light intensity (using a luxmeter) and mean leaf litter depth (using a ruler). All the measurements were taken in all the plots and the nursery. Measurements were taken across all plots and control areas at each evaluation, with averages reported. Results are shown in Table S3.

Soil samples were collected in triplicate from two points per sampling area. Analyzed parameters included pH, intensity of macronutrients (phosphorus and potassium), intensity of micronutrients (calcium and sulphur), organic matter content and physical composition of soil (silt, sand and clay). In the leaf litter, collected in single samples at two different points, the parameters for macronutrients (nitrogen, phosphorus and potassium) and micronutrients (calcium, magnesium and sulphur) were measured. Results are presented in Table S4.dividual performance metrics included survival (assessed visually: dead individuals showed dry appearance, brown foliage/structures, or complete defoliation), height (from ground level to apical meristem), and ground-level diameter (measured with calipers).

Data analysis

The R language was used to analyze the data using the RStudio graphical interface version 2022.12.0 (R Core Team, 2024; RStudio Team 2020).

Species establishment was assessed following the methodology of Atondo-Bueno et al. (2018). Survival was estimated using Kaplan-Meier analysis with the *survminer* (Kassambara et al. 2021) and *survival* (Therneau 2023) packages. Relative Growth Rate (RGR) was calculated for height and diameter using initial and final measurements.Height and diameter RGRs were visualized using boxplots generated with the *ggplot2* (Wickham 2016) and *ggthemes* (Arnold 2021) packages to compare distributions between enrichment techniques.

To evaluate the influence of environmental variables (vegetation, climate, soil, litter) on individual establishment, Principal Component Analyses (PCAs) were performed following Yeong et al. (2016), grouping parameters by variable type using the *factoextra* package (Kassambara & Mundt 2020). PCA biplots were created to assess relationships between axes and relative species abundances.

Multivariate differences between techniques were tested using Multivariate Analysis of Variance (MANOVA) with Pillai's trace test, implemented via the *permute* (Simpson 2022) and *lattice* (Sarkar 2008) packages, comparing relative abundance data against environmental variables and enrichment techniques.

Results

Emergence in direct sowing

A total of 216 emerging individuals were observed between the two areas (Table S5). Of the 151 emerging individuals in PNMGD, *E. uniflora* had the highest number of representatives (56 in the individual treatment and 42 in the Mix treatment). As for FAECA, of the 65 individuals, *E. uniflora* also had the highest number of emergents (15 in the individual treatment and 21 in the Mix treatment). In both areas, *A. urundeuva* was the species with the lowest number of emergences, and no emerged individuals were recorded in FAECA

At the control points in both areas, no emergence or presence of any individuals of the same species used in this study was observed.

Survival of individuals

In terms of the survival of individuals (Fig. 3A), seeding had a higher estimated survival of individuals, but no statistical difference whas found between techniques.

In direct seeding (Fig. 3B), *E. uniflora* was the species with the highest observed survival, while *H. courbaril* had an estimated survival of between 50 and 75%. As no *A. urundeuva* individuals survived, its estimate was based to the first data collection only.

For seedlings planting (Fig. 3C), *A. urundeuva* and *H. courbaril* had the highest estimated survivals, while *E. uniflora* was below 50%. When comparing Figs. 3B and 3C, only *H. courbaril* displayed similar estimated across both techniques.

[Figure 3 near here]

Growth of individuals

Figure 4 below shows the growth and development of sampled individuals, comparing the height and diameter RGRs of the individuals sampled.

Regarding diameter, seedlings planting exhibited only positive growth rates with greater variation, while direct seeding showed a relative rate of decay, but with diameters similar to planting seedlings. *Outliers* were observed in both techniques, with more in seedling planting.

For height, both techniques showed positive growth, but direct seeding exhibited significantly greater increases compared to seedling planting, which again had numerous outliers.

[Figure 4 near here]

Effect of vegetation, soil, leaf litter, environmental variables and treatments on the species

Figure 5 shows the PCA distribution of environmental variables influencing the abundance of sampled individuals. **[**Figure 5 **near here]**

In terms of environmental variables (Fig. 5A), light intensity was the only component with a positive correlation between PCA axes one and two, and this interaction was greater with sowing. Temperature and relative were related only to components one and two, respectively, with no interactions between the two techniques.

Observing the vegetation variables (Fig. 5B), both parameters exhibited negative correlations, with individual density showing the strongest negative correlation under direct seeding.As for the soil variables (Fig. 5C), since there were no differences in the values (sowing and planting seedlings), their distributions are superimposed. Positive relationships were observed’between organic matter and calcium with the individuals establishment, while sulfur had a negative correlation with axis two. The other components (phosphorus, potassium and pH) had positive correlations only with axis one.

Finally, in the litter variables (Fig. 5D), all the variables had positive interactions with at least one of the PCA axes, and these correlations were observed in both techniques.

Using the relative abundance of the surviving individuals, these were compared them with the PCAs of all the environmental variables and the enrichment techniques (Table 1). Significant differences were found only when comparing individual abundance between techniques.

Table 1
Comparison of the relative abundance of surviving individuals with the PCA components (environment, vegetation, soil, and litter) and enrichment techniques. Values with significant differences are shown in bold.

Variables	Df	Pillai	approx F	Num Df	den Df	Pr (> F)
PCA_ENVIRONMENT	3	0,61608	1,7229	9	60	0,10346
PCA_VEGETATION	2	0,19563	0,6866	6	38	0,66151
PCA_SOIL	6	0,77853	1,1682	18	60	0,31559
PCA_LITTER	7	0,50338	0,5761	21	60	0,91908
TECHNIQUES	1	0,37743	3,6374	3	18	**0,03277 ***
Code meanings: ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1						

[Table 1 near here]

Discussion

The low emergence rate observed in direct seedingsuggests the influence of various ecological filters, both biotic and abiotic (Azevedo et al. 2015). Among these factors, predation warrants special attention, particularly since evaluations began only three months after initial implementation. Authors such as Júnior et al. (2004) and Ferreira et al. (2007) highlight the significant impact of seed and herbivore predation on emerging individuals in direct seeding, limiting emergence and subsequent establishment of individuals.

The complete lack of *A. urundeuva*, despite its high viability, contrasts with literature findings. This species is known for its adaptability (Nunes et al. 2008) and has been extensively studiedregarding germination and establishment (Dorneles et al. 2005; Pacheco et al. 2006). As a negatively photoblastic species (Silva et al. 2002), the absence of emerged individuals suggests the possibility enviromental disturbances, warranting furthebinvestigations.

The emergence observed for *E. uniflora* and *H. courbaril* was expected, considering that both species were introduced into TSF environments with favorable physiological characteristics, as pointed out by Scalon et al. (2001) and Sousa et al. (2019). Notably, it should be noted that the tegument dormancy of *H. courbaril* (Fowler & Bianchetti, 2000) ensured its continuous establishment throughout the study. This pattern was also observed for *E. uniflora*, despite lacking seeddormancy. Lattuada and Souza (2011) note that delayed and uneven note that delayed and unevengermination of, is not necessarily due to dormancy, but rather to integument characteristics, which may impair seedling emergence.

The differences observed between direct seeding and seedlings planting techniques regarding individual survival indicate that not all species respond similarly during initial establishment (Moiron et al. 2019). Therefore, prior studies and analyses in target areas for assisted enrichment are highly recommended to enhance technique effectiveness, as suggested by Tarou et al. (2007) and Yeong et al. (2016).

These differences, while being natural due to each technique's distinct premises, also highlight the need for alternatives to address encountered challengesliterature-highlighted possibilities, combining seedling planting and direct seeding shows promise (Silva et al. 2020; Palma & Laurace 2015). This integrated approach ensures rapid establishment of key species while promoting greater diversity at restoration sites.

Seedlings planting guarantees initial establishment of key species, while direct seeding enhances diversity and facilitates natural regeneration. Thus, combiningthese techniques representsmethod an effective strategy for restoring degraded areas, overcoming individual limitations.

The observed survival discrepancies across techniques underscorethe importance of careful species selection for assisted enrichment, as discussed by Sampaio et al. (2021). For example, *A. urundeuva* and *E. uniflora* contrasting performance: high survival in one technique but poor performance in the other.

It was observed that *H. courbaril* showed good adaptation to its habitat, especially with regard to establishment and survival in both cultivation techniques. However, a study conducted by Mangueira et al. (2018) reported contrasing results, with direct seeding sowing having significantly higher survival and establishment rates compared to planting seedlings. These discrepancies may result from species adaptability and environmental stresses, as discussed by Nawaz et al. (2023).

Despite the similarities in the height and diameter measurements, direct seeding exhibited greater relative growth than seedlings planting throughout the study period. However, Atondo-Bueno et al. (2018) warn caution that comparing species at different development stages may be inappropriate due to the varying levels affecting individuals and influencing results.

The significant growth observed in direct sowing was expected, given this technique's characteristics that favor the adaptation environmental stress and select for the most resilient individuals (Sampaio et al. 2015). Furthermore, considering species-specific adaptation to local conditions is crucial for success, as emphasized by Coradin et al. (2018), Nascimento et al. (2022), and Melo et al. (2015).

The parity of growth metrics in seedling planting results from the presence of already developed organs (COSTA et al., 2020), such as the root system, which provides greater environmental adaptation (GROSSNICKLE & IVETIC 2022) and reduces the disturbances impacts, leading to uniform plant growth (MARERI et al., 2022).

Negative RGRs indicates a deceleration in individual growth rates. This decrease does not necessarily implies a reduction of the initial size but a small growth. (Hunt 1990). This phenomenon arises from the equation used, where growth variation below 1 yields negative RGR values

Analysis of light intensity diversity and its relationship with tree canopy revealed etiolation signs in direct-seeded seedlings. This phenomenon, characterized by accentuated vertical growth, affects structural development such as root formation (Salton & Tomazi 2014) and physiological processes (Hartmann & Kester 1990), though commonly observed in forest environments. Photoperiod influence in these contexts favors inter-individual competition (Câmara et al. 1997; Basler & Körner 2014).

The positive correlation observed between macronutrients and micronutrients in the litter directly related to TSF intrinsic characteristics, known for high fertility and nutrient availability (Bauer et al. 2017). In such forests, this dense organic residue layer plays a crucial role in plant establishment (Bastos et al. 2021), promoting nutrient cycling and soil protecting (Pinto et al. 2009).

Although soil nutrient levels were adequate, no direct relationship was found between individual abundance and techniques used. This lack of association primarily results from data discrepancies between sampled areasHowever, this does not imply minimal soil nutrition influence on individuals; rather, differences were not statistically significant during the sampling period (Vezzani & Mielniczuk 2009)

Although no statistically significant differences were identified among environmental parameters, this absenceof evidence does not allow us definitive dismissal of their influence on initial establishment. From this perspective, establishment success appears more related to species intrinsic characteristics (Vieira et al. 2022), which played a crucial role in observed results.The tudied species are known for adapting to diverse environments and conditions, facilitating establishment even in disturbed areas (Silva 2024). This adaptive capacity stems from intrinsic traits such as generalist behavior and native origin, enabling exploitation of various ecological niches (Kirsch & Kaproth 2022).

Declarations

Aditional information

The author(s) declare no competing interests.

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Author Contribution

JVLP originally conceived the idea; JVLP contributed to study design; JVLP e JDD collected the data; JVLP e ZVP analyzed the data; JVLP wrote the first draft of the manuscript with advice from ZVP; all authors contributed critically to the drafts and gave final approval for publication.

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Data Availability

The data that support the findings of this study are openly available in GITHUB at https://github.com/joaoDINO21/ENRICHMENT.git,reference number DICE 1.

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Figures

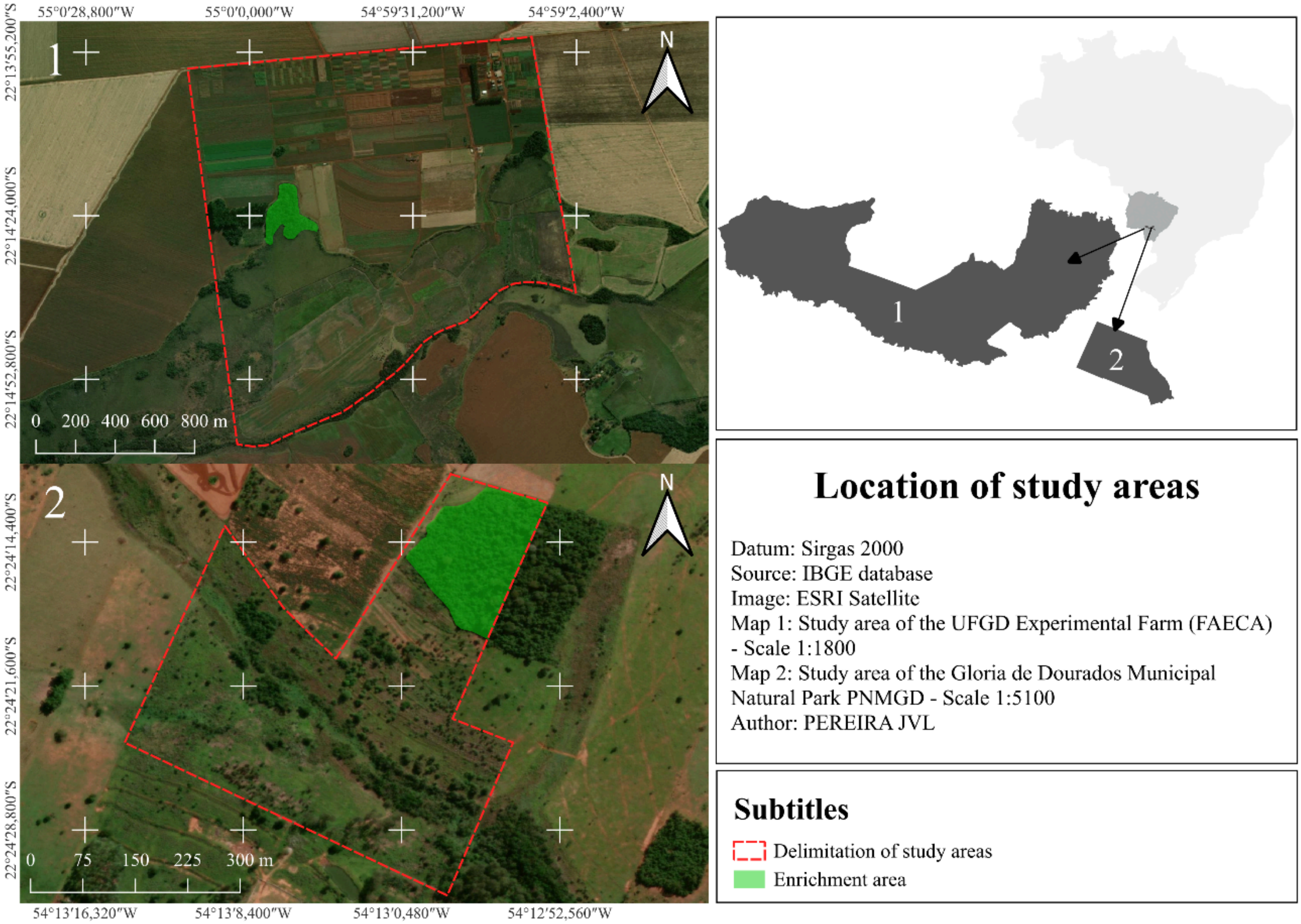

Figure 1

Map showing the locations of the experimental areas of PNMGD and FAECA.

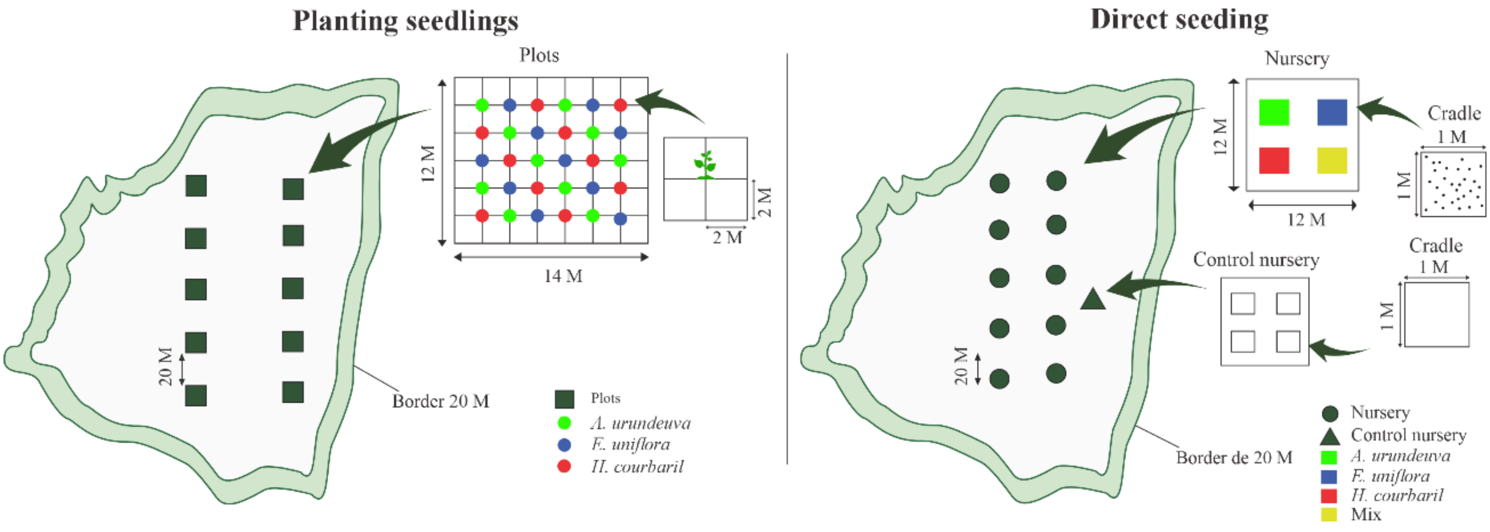

Figure 2

Sketch representing the sampling design used in the study.

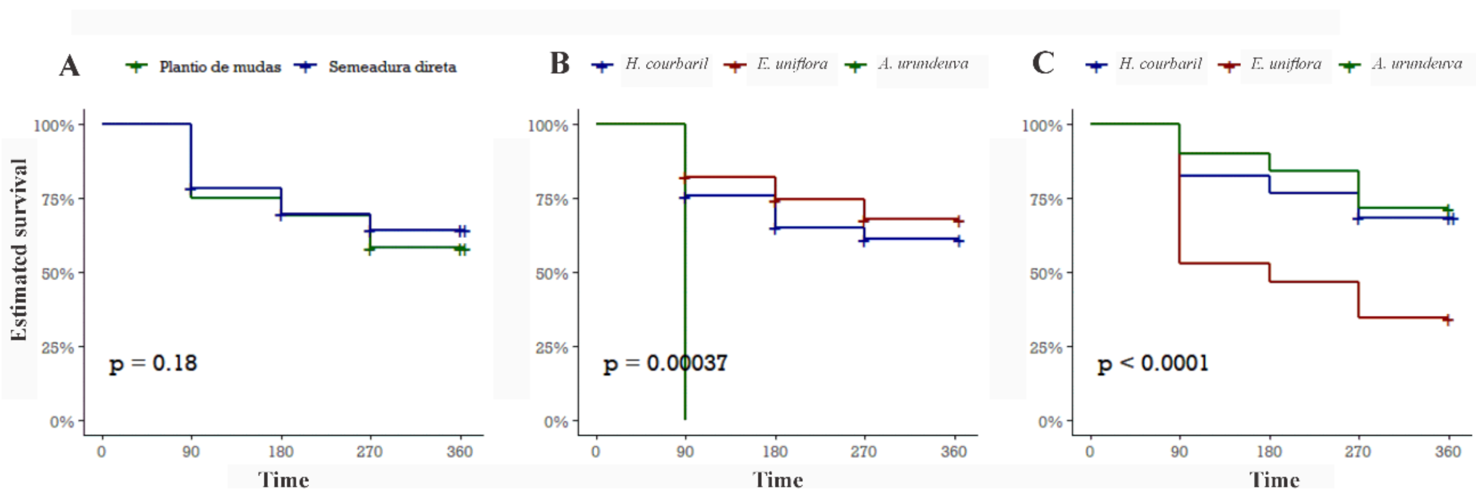

Figure 3

Graphical representation of the estimated survival of individuals, comparing the estimated survival among techniques (**A**), the estimated survival in the direct seeding technique only (**B**), and the estimated survival in seedling planting only (**C**).

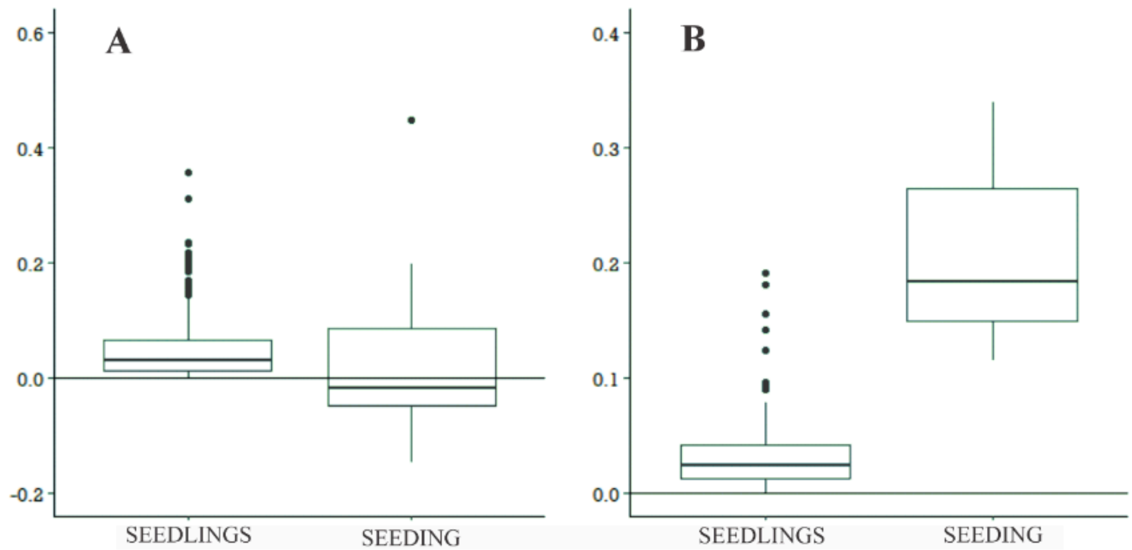

Figure 4

Graphical representation comparing the RGRs of sampled individuals using enrichment techniques, showing the diameter of individuals (**A**) in mm·mm⁻¹·month⁻¹ and height (**B**) in cm·cm⁻¹·month⁻¹.

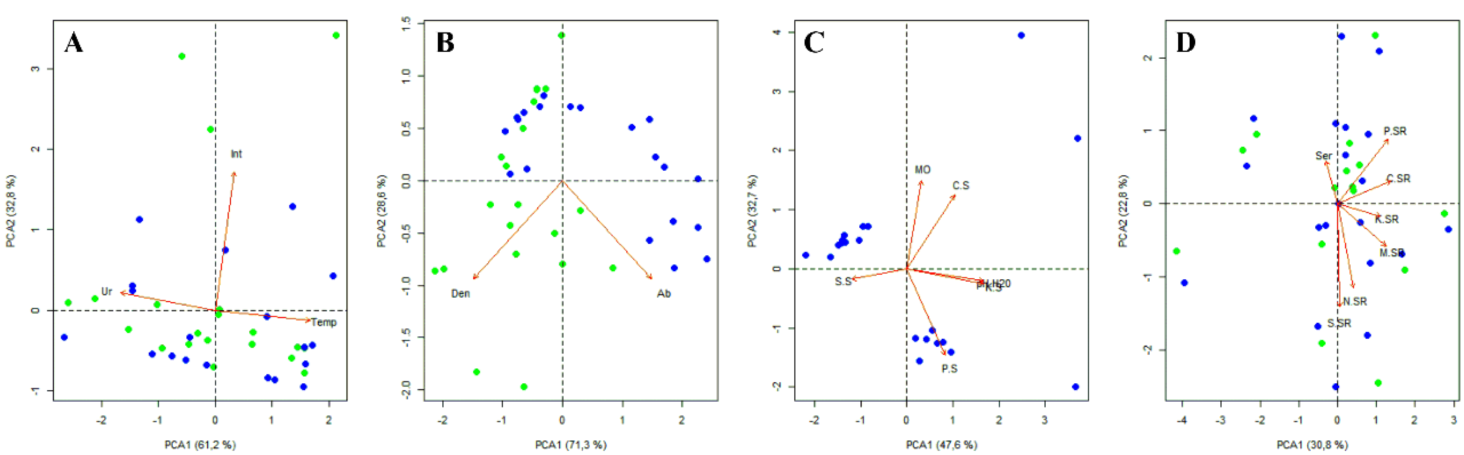

Figure 5

Graphical representation of the PCA components of environmental variables (**A**), vegetation (**B**), soil (**C**), and litter (**D**). Abundance of individuals related to seedling planting is shown in green, and that related to direct seeding is shown in blue.

Supplementary Files

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