Research Article
doi: 10.1590/01047760202531013503
vol(31), 2025

CERNE

Effectiveness of direct seeding techniques for forest restoration in a Brazilian Cerrado area

Paolo Alessandro Rodrigues Sartorelli[1], Andressa Ribeiro[2✤], Gabriel Marx Cordeiro[1], Mauricio Sangiogo[3], Kennedy de Paiva Porfírio[2], Antonio Carlos Ferraz Filho[2]

[1]Baobá Florestal, Osasco, SP, Brazil
[2]Federal University of Piauí, Campus Bom Jesus, Bom Jesus, PI, Brazil
[3]Fundação Estadual de Proteção Ambiental Henrique Luiz Roessler, Porto Alegre, RS, Brazil

SILVICULTURE

ABSTRACT

Background: Forest restoration plays a crucial role in mitigating climate change. In the Cerrado biome, effective and scalable restoration techniques are still lacking, particularly those that balance logistics, cost, and ecological success. This study aimed to evaluate the effectiveness of four direct seeding techniques for forest restoration under field conditions.

Results: The tested treatments included: (DS) direct seeding with native species, (DS+BAC) with a bacterial consortium, (DS+NPK) with NPK fertilizer, and (DS+BAC+NPK) with both inputs. The experiment, conducted in Monte Alegre de Minas (MG, Brazil), used a randomized complete block design with 16 plots. Botanical inventories occurred at 63, 105, 164, and 454 days post-sowing. Significant height differences were observed, with DS+BAC+NPK yielding the tallest plants. Species richness showed no consistent differences across treatments, while diversity and evenness increased over time. Similarity among plots declined, indicating vegetation differentiation among treatments.

Conclusion: All four direct seeding techniques showed promise for Cerrado restoration, but DS+BAC+NPK was the most effective in promoting plant growth and ecological indicators. Continued long-term monitoring is essential to fully understand the role of all four direct seeding techniques showed promise for Cerrado restoration, although DS+BAC+NPK was the most effective in promoting plant growth and ecological indicators. Continued long-term monitoring is essential to fully understand the role of forest restoration in various ecological contexts.

Keywords: Bacterial consortium; Cerrado restoration; climate change; forest inventory; native species.

HIGHLIGHTS

Forest restoration techniques that consider bacterial consortium are new in forestry.
Plant height showed significant differences between evaluated treatments.
Diversity and evenness increased over time, with a decline in similarity.
The best technique was direct seeding plus bacterial consortium and fertilizer.

SARTORELLI, P. A. R.; RIBEIRO, A.; CORDEIRO, G. M.; SANGIOGO, M.; PORFÍRIO, K. P.; FERRAZ FILHO, A. C. Effectiveness of direct seeding techniques for forest restoration in a Brazilian Cerrado area. CERNE, v.31, e-103503, 2025. doi: 10.1590/01047760202531013503

✤Corresponding author: andressa.florestal@ufpi.edu.br
Scientific Editor: Gilvano Ebling Brondani

Received: November 8/2024
Accepted: May 12/2025

CERNE
ISSN 0104-7760
UNIVERSIDADE FEDERAL DE LAVRAS

INTRODUCTION

Climate change is a reality in the era recently called the Anthropocene. Despite the long history of environmental degradation by human activities, only recently has research been developed, such as restoration ecology, to understand the dynamics of these processes and to attempt to revert them (Cooke et al., 2019; Guerra et al., 2020). The Paris Agreement of the United Nations Framework Convention on Climate Change entered into force in 2016 with the objective of strengthening the global response to climate change. One of Brazil's nationally determined contributions (NDC) included the target of restoring and reforesting 12 million hectares of forests for multiple uses by 2030 (Bustamante et al., 2019).

The Brazilian Cerrado is one of the most biodiverse savannas in the world, yet 46% of its original cover has been converted to crops and pastures, and despite the global importance of savanna biomes, international and domestic conservation efforts tend to prioritize rainforests (Rodrigues et al., 2022). Researchers has already been conducted in the Cerrado biome with the aim of restoration; highlighting studies using direct seeding with native trees (Silva and Vieira, 2017; Oliveira et al., 2019; Giles et al., 2022), studies including sampling of herbaceous, shrub, herb and grass species (Pellizzaro et al., 2017; Coutinho et al., 2018; Sampaio et al., 2019; Oliveira et al., 2020), evaluating techniques such as the transposition of topsoil and hey (Ferreira et al., 2015; Pilon et al., 2017, 2018). Other studies analyzed different sampling methods for restoration indicators (Chaves et al., 2015; Silva and Vieira, 2017), costs of restoration techniques (Palma and Laurance, 2015; Brancalion et al., 2019; Raupp et al., 2020) and supply networks of native seeds and seedlings for restoration projects in the Cerrado biome (Schmidt et al., 2019; Silva et al., 2022).

However, an effective answer for the ambitious plans for forest restoration at global level is still unknown. It is imperative to use the most suitable establishment technique directed at both ecological and economic aspects of the restoration process (Grossnickle and Ivetić, 2017). The recent debate has revolved around how restoration projects should be conducted. According to Matzek et al. (2017), these projects should aim to recreate the historic species assemblage in an area to the greatest extent possible, or should restoration efforts focus on creating ecosystems that will be functional and dynamic in the future? Traditionally, forest restoration has aimed at recovering the same or very similar conditions of the original forest (Medeiros et al., 2022). This has mostly been achieved through active revegetation, natural regeneration, or mixed approaches. These methods can include planting seedlings of native and/or exotic species, natural regeneration, assisted natural regeneration, or establishing agroforestry systems (Cruz et al., 2021). Active restoration, which involves planting a high number of tree species, is the most used, recommended, and developed restoration method for deforested areas (Raupp et al., 2020; Cruz et al., 2021).

Ecological restoration still presents substantial challenges for tropical and megadiverse countries. Brazil has a challenge to address plans that are technically and financially feasible, public policies and monitoring instruments that can assess effectiveness diagnosis of the area, based on the ecosystem needs (Bustamante et al., 2019). There is an existent lack of rigorous analyses about the major components and drivers of restoration costs limits the development of alternatives to reduce costs and the selection of the most cost-effective methods to achieve restoration goals (Brancalion et al., 2019).

As an alternative to planting of seedlings, direct seeding technique has been recommended for forest restoration due to its lower cost and versatility and for allows the introduction of different plant growth forms simultaneously (Palma and Laurance, 2015; Grossnickle and Ivetić, 2017; Pellizzaro et al., 2017; Raupp et al., 2020). The method of direct seeding involves mixing native seed species, fast-growing legumes, and a filler material, typically sand, to homogenize the seeds for sowing (Campos-Filho et al., 2013; Raupp et al., 2020).

Given Brazil's commitment to restoring degraded areas and the significant increase in deforestation and fragmentation in the Cerrado biome (Pompeu et al., 2024), it is crucial to study optimal techniques for the restoration process. These techniques include direct seeding and the use of various inputs such as bacterial consortium and fertilizers. Therefore, the objective of this work was to monitor the restoration process in a Cerrado area in Minas Gerais state, evaluating four direct seeding techniques and testing the hypothesis that incorporating a bacterial consortium (*Bacillus* spp., *Pseudomonas* spp., *Lysinibacillus* spp., *Azospirillum* spp.) and NPK fertilizer enhances the restoration process.

MATERIAL AND METHODS

Experimental design

The experimental area was established in November 2022 in the municipality of Monte Alegre de Minas, Minas Gerais (Figure 1). The region is classified as Aw (hot with rainy summer) according to the Köppen's climate classification, with an average annual precipitation of 1500 mm (Alvares et al., 2013). The original vegetation physiognomy was in the transition from Cerradão to Semi Deciduous Seasonal Forest (IBGE, 2024), with field observations revealing remnant individual's characteristic of both formations, such as *Plathypodium elegans* (Fabaceae), *Annona coriacea* (Annonaceae), *Bowdichia virgiliodes* (Fabaceae), *Scheflera morotononi* (Araliaceae), and *Myracruodruon ururundeuva* (Anacardiaceae).

Before the sowing, the land was conventionally prepared using a tractor with a 20 cm disc harrow attached and lime was incorporated throughout the area (3 t ha^{-1}). A composite sample soil analysis was conducted, and the following contents were obtained: 80.9% of sand, 18.1% of clay and 1.0% of silt. The chemical results were: 5.28 pH in water, 16.9 mmolc dm^{-3} of calcium, 5.5 mmolc dm^{-3} of magnesium, 1.3 mmolc dm^{-3} of potassium, 2.2 mmolc dm^{-3} of aluminum, 1.8 mg dm^{-3} of phosphorus, 11.04 g dm^{-3} of organic matter, 45.89 mmolc dm^{-3} of cation exchange capacity, and 51.86% of base saturation.

The experimental design adopted was a randomized complete block with four treatments and four replications, with 4 ha in each block. The sample plot's dimensions were 8 x 25 m, totaling 16 plots (Figure 1). The analyzed treatments were: (DS) - direct seeding with forest species from Cerrado biome, (DS+BAC) - direct seeding plus bacterial consortium (mix of *Bacillus* spp., *Pseudomonas* spp., *Lysinibacillus* spp., *Azospirillum* spp. with final concentration of 1x107 UFC g^{-1} and incorporation of 8 kg ha^{-1}), (DS+NPK) - direct seeding plus NPK fertilizer (04-14-08 formulation and incorporation of 300 kg ha^{-1}) and, (DS+BAC+NPK) - direct seeding plus bacterial consortium and NPK fertilizer, in the same quantities as described in the previous treatments.

Sowing and Data collection

An average of 67 kilograms of seeds per hectare (±15 kg ha^{-1}) was sown in each treatment. The seeds varied in size and were collected from different locations, ensuring their vitality, good health, and provenance. Direct seeding was carried out mechanized using a solid distribution implement attached to a tractor and broadcast, followed by soil incorporation using a leveling harrow. This methodology is the same as that used for the distribution of limestone and NPK. It should be noted that all the seeds were collected and stored in a natural environment within a shed, and the germination viability was not assessed before the direct seeding process. The seed mixture was composed by 12 botanical families (46% Fabaceae), totaling 57 species of Cerrado flora for forest restoration purposes (Table 1), as proposed by Campos Filho et al. (2013); Campos Filho and Sartorelli (2015). These species were also chosen due to their natural occurrence in the dominant vegetation type near the area.

Data analysis

Data were collected during four inventories carried out in the sample plots with 63, 105, 164 and 484 days after sowing (Figure 2). All woody plants were counted, and their heights measured using a ruler graduated in centimeters. Each plant was botanically identified to the species level when possible, or to the genus level if species identification was not feasible. Plants that could not be identified were classified as NI (Not Identified).

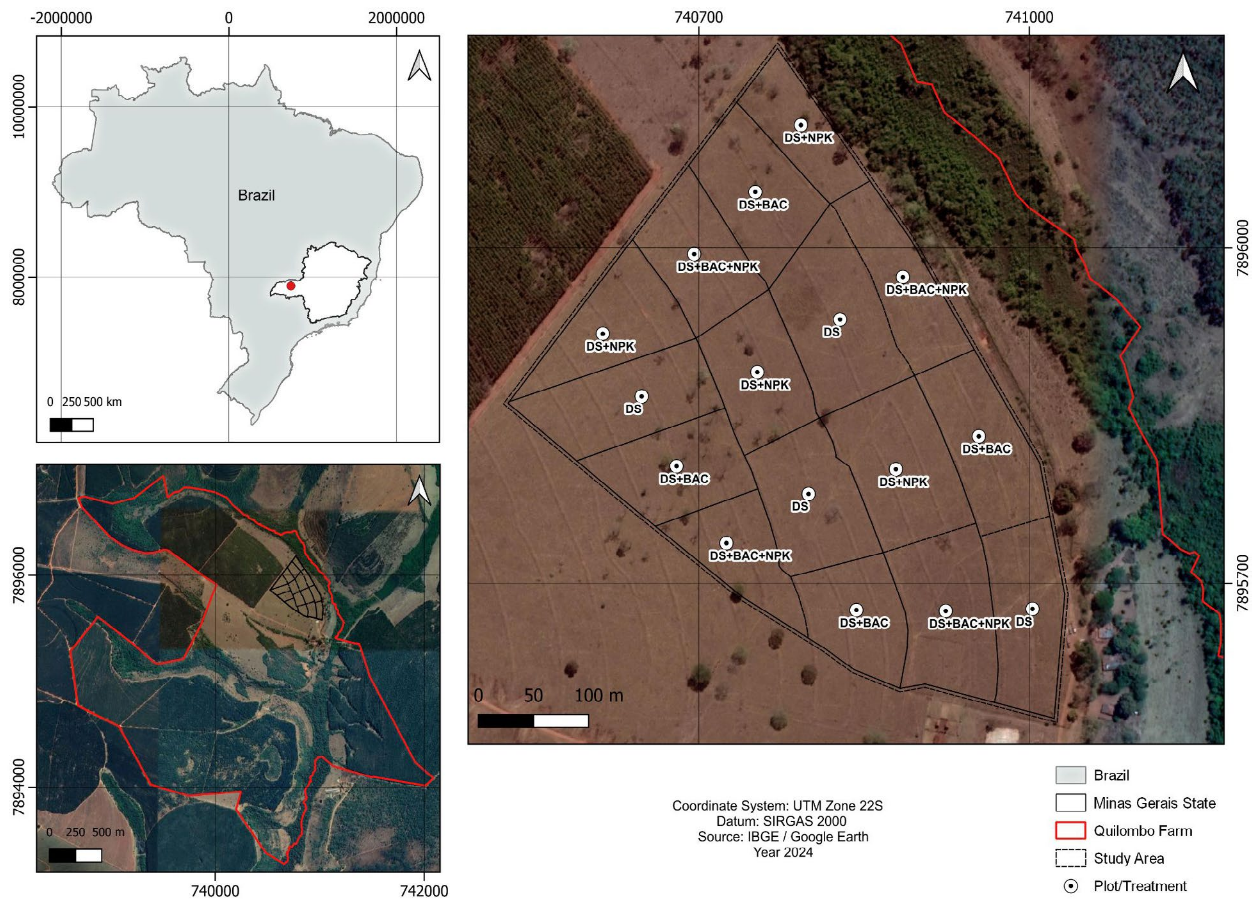

Figure 1: Location of a forest restoration experiment in a Cerrado area to evaluate direct seeding techniques.

Table 1: Species sowed for evaluation of direct seeding techniques in a forest restoration experiment in a Cerrado area, located in Monte Alegre de Minas, MG.

Scientific name	Botanic family
Anacardium humile A.St.-Hil.	Anacardiaceae
Anacardium occidentale L.	Anacardiaceae
Anadenanthera colubrina (Vell.) Brenan	Fabaceae
Apeiba tibourbou Aubl.	Malvaceae
Apuleia leiocarpa (Vogel) J. F. Macbr.	Fabaceae
Astronium fraxinifolium Schott	Anacardiaceae
Astronium urundeuva (M.Allemão) Engl.	Anacardiaceae
Bixa orellana L.	Bixaceae
Byrsonima basiloba A. Juss.	Malpighiaceae
Byrsonima crispa A. Juss.	Malpighiaceae
Byrsonima cydoniifolia A. Juss.	Malpighiaceae
Byrsonima intermedia A.Juss.	Malpighiaceae
Byrsonima verbascifolia (L.) DC.	Malpighiaceae
Ceiba speciosa (A. St.-Hil.) Ravenna	Malvaceae
Chloroleucon acacioides (Ducke) Barneby & J.W.Grimes	Fabaceae
Copaifera langsdorffii Desf.	Fabaceae
Copaifera martii Hayne	Fabaceae
Dipteryx alata Vogel	Fabaceae
Enterolobium contortisiliquum (Vell.) Morong	Fabaceae
Enterolobium maximum Ducke	Fabaceae
Eriotheca pubescens (Mart.) Schott & Endl.	Malvaceae
Erythrina speciosa Andrews	Fabaceae
Guazuma ulmifolia Lam.	Malvaceae
Hymenaea courbaril L.	Fabaceae
Hymenaea stigonocarpa Mart. ex Hayne	Fabaceae
Jacaranda brasiliana (Lam.) Pers.	Bignoniaceae
Jacaranda micrantha Cham.	Bignoniaceae
Mabea fistulifera Mart.	Euphorbiaceae
Mimosa bimucronata (DC.) Kuntze	Fabaceae
Peltophorum dubium (Spreng.) Taub.	Fabaceae
Platypodium elegans Vogel	Fabaceae
Pseudobombax grandiflorum (Cav.) A.Robyns	Malvaceae
Pseudobombax longiflorum (Mart. & Zucc.) A. Robyns	Malvaceae
Pseudobombax tomentosum (Mart. & Zucc.) A. Robyns	Malvaceae
Psidium guajava L.	Myrtaceae
Pterogyne nitens Tul.	Fabaceae
Schizolobium parahyba (Vell.) Blake	Fabaceae
Senegalia polyphylla (DC.) Britton & Rose	Fabaceae
Senna alata (L.) Roxb.	Fabaceae
Senna multijuga (Rich.) H.S.Irwin & Barneby	Fabaceae
Senna spp.	Fabaceae
Solanum crinitum Lam.	Solanaceae
Solanum lycocarpum A.St.-Hil.	Solanaceae
Solanum mauritianum Scop.	Solanaceae
Solanum sp.	Solanaceae
Sterculia striata A.St.-Hil. & Naudin	Malvaceae
Stryphnodendron adstringens (Mart.)	Fabaceae
Stryphnodendron rotundifolium Mart.	Fabaceae
Syagrus romanzoffiana (Cham.) Glassman	Arecaceae
Tachigali vulgaris L.G.Silva & H.C.Lima	Fabaceae
Tamarindus indica L.	Fabaceae
Vernonanthura polyanthes (Sprengel) Vega & Dematteis	Asteraceae

Continue...

Table 1: Continuation.

Data of height (in centimeters), number of species (richness), abundance (density), diversity (Shannon-Wiener index - H’), evenness (Pielou index - J’) and similarity (Sorenson index - Ss) were appropriately tabulated and analyzed by treatment and measurement. Here, abundance was defined by the number of individuals on the same sample plot and the density was calculated according to equation (1), which measures the number of target species per given area. The species ∝-diversity is usually described by two basic constituents (richness and evenness), here we adopted the Shannon-Wiener diversity index, calculated by equation (2). The evenness is represented by the Pielou index (J’) and was calculated according to equation (3). To estimate β-diversity we calculated the similarity by Sorensen index - Ss, according to equation (4).

$$Density = \frac{(number\ of\ measured\ individuals)}{(surface\ area\ of\ sample\ unit)} \quad (1)$$

$$H' = -\sum_{i=1}^{S} p_i (\ln p_i) \quad (2)$$

where: p_i is the proportion of individuals belonging to the ith species and S is the total number of species.

$$J' = H' / H'max \quad (3)$$

where: H’ is the number derived from the Shannon-Wiener diversity index and H’max is the maximum possible value of H’ (if every species was equally present). H’max= ln(S), where S is the total number of species.

$$Ss = 2c / (a + b) \quad (4)$$

where: *c* is the number of species shared by both plots and *a* and *b* is the number of species in each plot/treatment.

All statistical analysis was conducted using the R statistical environment (R Core Team, 2021) at 95% of significance level. One-way ANOVA test was performed for height and density values during the period of monitoring, and multiple comparison was conducted by Tukey's HSD test with the *HSD.test* function from *agricolae* package (Mendiburu, 2023). Previously, the assumptions of ANOVA and prospecting for outliers were analyzed, and data of height were transformed by log10(×). The variable richness was fitted to a generalized linear model (GLM) with Quasi-Poisson distribution, and multiple comparison was conducted by *ghlt* function from the *multcomp* package (Hothorn et al., 2008). Shannon-Wiener diversity index was compared using the Hutchenson t-test, calculated by the function *multiple_Hutcheson_t_test* of the *ecolTest* package (Salinas and Ramirez-Delgado, 2021). Sorensen index is presented as a matrix for each treatment and measurement, which varies from 0 (dissimilarity) to 1 (total similarity).

RESULTS

A total of 69 tree species were found during the four measurements, 23 botanic families with Fabaceae and Bixaceae being the most representative, computing together more than 80% of the species. The analysis of variance for plant height values during the monitoring time revealed statistically significant differences in treatments (p value < 0.001). Tukey's HSD test was performed to differentiate the averages (Figure 3A) showing that the DS+BAC+NPK treatment resulted in the tallest plants 454 days after sowing, although it differed statistically only from the DS treatment. Species richness did not display significant differences between treatments over time ($p > 0.05$), except for the first measurement taken 63 days after sowing. During this period, there was a statistically significant difference between treatments ($p = 0.041$), with the DS+BAC+NPK treatment showing superior values (Figure 3B). Density values also did not show significant differences between treatments over time ($p > 0.05$), with the highest average of 16,369 plants per hectare recorded at the third measurement, 164 days after sowing (Figure 3C).

Although the values of richness and density did not show significant differences between the treatments, when analyzing the values at 484 days after sowing, it is notable the superiority of DS+BAC+NPK treatment among others, especially for density values, reaching 19,850 plants against 11,413 for the lowest treatment (DS+BAC). The species *Apuleia leiocarpa*, *Bixa orellana*, *Enterolobium contortisiliquum*, *Schizolobium parahyba*, *Senna alata*, *Solanum* sp. and *Sterculia striata* were the most frequent for all treatments over time.

The results for diversity, evaluated using the Shannon-Wiener index (H'), and evenness, assessed by the Pielou index (J'), showed a tendency to increase over time for all treatments, with the exception of direct seeding plus fertilizer (DS+NPK). The ranking of the results varied during time, and the highest values after 484 days of sowing occurred in the direct seeding treatment with bacterial consortium (H' = 2.42 and J' = 0.35), followed by DS > DS+BAC+NPK > DS+NPK. The Hutcheson test showed significant differences for some direct seeding techniques, especially 484 days after sowing the significant differences that were more prominent between the treatments ($p < 0.005$, Table 2), except for DS compared to DS+BAC.

The results from the Sorensen similarity index did not indicate any notable tree species occurrence dissimilarities between the treatments and measurements (Table 3), with treatment DS+BAC+NPK and DS+BAC being more similar for most of the inventories. Considering all the direct seeding techniques, the mean value of the Sorensen similarity index decreases during the measurements, from 0.87 (63 days after sowing) to 0.78 (484 days after sowing).

Figure 2: Preparing the material for forest restoration (A), mechanized direct seeding (B), experimental area 63 days (C), 105 days (D), 164 days (E) and 484 days after sowing (F) in Monte Alegre de Minas, MG.

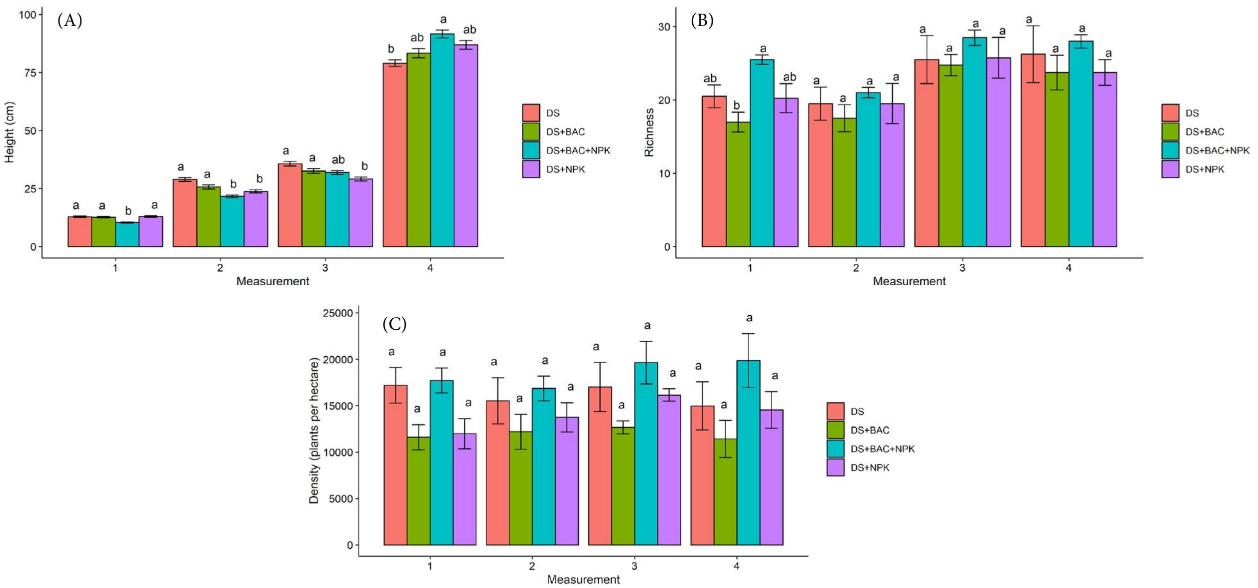

Figure 3: Tukey's HSD test for height (A), richness (B) and density (C) average values for the evaluated direct seeding techniques in a forest restoration in a Cerrado area, located in Monte Alegre de Minas, MG. Where measurement 1 = 63, 2 = 105, 3 = 164 and 4 = 484 days after sowing. Bars represent the standard error of the mean.

Table 2: Values of Shannon-Wiener diversity index (H') and Pielou evenness index (J) and *p* values of Hutcheson test to compare H' in direct seeding techniques for a forest restoration experiment in a Cerrado fragment, located in Monte Alegre de Minas, MG.

63 days after sowing	H'	J	Hutcheson test (p values)		
			DS	DS+BAC	DS+BAC+NPK
DS	2.01	0.28			
DS+BAC	2.16	0.32	0.0025**		
DS+BAC+NPK	2.08	0.32	0.0954^{ns}	0.0833^{ns}	
DS+NPK	2.17	0.29	0.0021**	0.4104^{ns}	0.0638^{ns}
105 days after sowing	**H'**	**J**	**DS**	**DS+BAC**	**DS+BAC+NPK**
DS	2.14	0.30			
DS+BAC	2.11	0.31	0.2754^{ns}		
DS+BAC+NPK	2.02	0.28	0.0089**	0.0394*	
DS+NPK	2.06	0.29	0.0569^{ns}	0.1585^{ns}	0.2439^{ns}
164 days after sowing	**H'**	**J**	**DS**	**DS+BAC**	**DS+BAC+NPK**
DS	2.31	0.32			
DS+BAC	2.33	0.34	0.3331^{ns}		
DS+BAC+NPK	2.08	0.30	0.0000**	0.0000*	
DS+NPK	2.17	0.28	0.0096**	0.0048**	0.0666^{ns}
484 days after sowing	**H'**	**J**	**DS**	**DS+BAC**	**DS+BAC+NPK**
DS	2.39	0.34			
DS+BAC	2.42	0.35	0.3259^{ns}		
DS+BAC+NPK	2.25	0.31	0.0090**	0.0036**	
DS+NPK	2.15	0.30	0.0001**	0.0000**	0.0436*

^{ns} not significant; * significant at 5% of probability; ** significant at 1% of probability.

DISCUSSION

Cerrado is a grassland-savanna-forest complex, and restoring millions of hectares of degraded mosaics is a daunting task (Schmidt et al., 2019). To ensure successful results in restoration projects, it is crucial to carefully consider the characteristics of the original vegetation. The presence of grasses, herbs, shrubs, and trees contributes to varied growth forms and species diversity, potentially fostering a complex native community (Pellizzaro et al., 2017). Recognizing that our current findings represent an early phase of the restoration process, and had just focus on tree species, we acknowledge the necessity for long-term monitoring to substantiate our hypotheses. This entails incorporating additional data points such as diameter measurements, survival rates, biomass analysis, soil studies, and expanded sampling of floral components.

Forest biodiversity is fundamental to maintaining healthy forest ecosystems and provision of multiple ecosystem services, the protection of forest biodiversity becomes more and more important in political and economic decision-making processes (Storch et al., 2023). Thus, the importance of testing different techniques for forest restoration is necessary to achieve success. For several decades, bacteria have been introduced into soil to improve plant growth (Garcí a et al., 2004) and few studies have tested it in the forest restoration process (Costa and Melloni, 2019; Ledea-Rodríguez et al., 2020; Abreu et al., 2021; Trápani et al., 2021), but it is common for agricultural species such as maize (Molina-Romero et al., 2021) and soybean (May et al., 2021). Strains of bacteria belonging to the genus *Pseudomonas*, *Azotobacter*, *Bacillus*, *Rhizobium*, *Azospirillum*, among others, have been widely used in agriculture, as growth promoters, increasing plant biomass and controlling plant diseases through different mechanisms (de Mandal et al., 2018; Debasis et al., 2019).

When analyzing overall plant height (Figure 3A), we found for the last measurement (454 days = 1.4 years after sowing) an average of 86 cm (±62 cm). Pellizzaro et al. (2017) evaluated the initial establishment success under field conditions of Cerrado species and reported that after the first rainy season (6 months after sowing) an average height of 7.2 ± 5.9 cm, and after the second rainy season (1.5 years after sowing) increasing to 10.1 ± 8.2 cm. Values of height differs significantly between biomes and restoration techniques, so we believe that the values from our study is satisfactory, since Cerrado includes different vegetation types as *campo limpo* (grassland with no trees), *campo sujo* (grassland with a few shrubs and small trees), *campo cerrado* (average tree height of 2–4m, no continuous canopy), *cerrado sensu stricto* (average tree height of 3–6m, no continuous canopy) and *cerradão* (average tree height of 8–15m) (Medeiros et al., 2022). The species *Bixa orellana*, *Mimosa paludosa*, *Schizolobium parahyba* and *Senna alata* were the tallest ones after 454 days of sowing, presenting some individuals higher than 3 meters.

Density values varied over time (Figure 3C) with values superior to 11,000 individuals. Average values for all measurements were 18,516 N ha^{-1} for DS+BAC+NPK treatment, followed by 16,178 N ha^{-1} for DS treatment, 14,103 N ha^{-1} for DS+NPK treatment and 11,966 N ha^{-1} for DS+BAC treatment. The species *Bixa orellana* (6,143 N ha^{-1}), *Senna alata* (2,206 N ha^{-1}), *Senna* sp. (1,376 N ha^{-1}) and *Solanum* sp. (901 N ha^{-1}), together correspond to 70% of the total density of the area. These values are in accordance with other studies carried out in Cerrado biome (Barreira et al., 2002; Silva et al., 2005; Campos-Filho and Sartorelli, 2015) High seedling density values can be a result of high seeding densities that contribute to quickly establishing the canopy (Raupp et al., 2020), therefore it is difficult to draw comparisons when using different methodologies.

In our study we focused only on woody plant individuals and found the highest richness values at the third monitoring (164 days after sowing), with exception of DS treatment, which presented the highest value 454 days after sowing (Figure 3B). The richness values found 1.4 years after sowing were 43 (DS treatment), 38 (DS+NPK treatment) and 42 (DS+BAC+NPK and DS+BAC treatment) woody species. According to Medeiros et al. (2022) the richness values of plant species used in assisted ecological restoration of degraded areas varied among Cerrado vegetation types *campo cerrado*

Table 3: Values of Sorensen similarity index (Ss) in direct seeding techniques for a forest restoration experiment in a Cerrado fragment, located in Monte Alegre de Minas, MG.

63 days after sowing	**DS**	**DS+BAC**	**DS+BAC+NPK**
DS+BAC	0.81		
DS+BAC+NPK	0.86	1.00	
DS+NPK	0.91	0.81	0.83
105 days after sowing	**DS**	**DS+BAC**	**DS+BAC+NPK**
DS+BAC	0.83		
DS+BAC+NPK	0.90	0.96	
DS+NPK	0.90	0.86	0.90
164 days after sowing	**DS**	**DS+BAC**	**DS+BAC+NPK**
DS+BAC	0.85		
DS+BAC+NPK	0.81	0.88	
DS+NPK	0.87	0.85	0.80
484 days after sowing	**DS**	**DS+BAC**	**DS+BAC+NPK**
DS+BAC	0.80		
DS+BAC+NPK	0.75	0.78	
DS+NPK	0.77	0.78	0.83

(10 plant species), *cerradão* (88 plant species), *cerrado sensu lato* (68 plant species), *cerrado sensu stricto* (179 plant species), ironstone outcrop (2 plant species), quartzitic rupestrian grassland (26 plant species), riparian forest (191 plant species), *mata seca* (21 plant species) and *vereda* (72 plant species).

It is a challenge to determine which seedling comes from natural regeneration or direct seeding technique during field campaigns, as well as to proceed with botanical identification for young plants. We noticed a change in density values from measurement 1 to 4, for example, *Hymenaea* sp. presented high density values until the third measurement, with a change to *Hymenaea stigonocarpa* and *Hymenaea courbaril* at the 4 measurements, since it was possible the botanical identification in species level for older plants. Therefore, inferences about diversity must be made with caution, and long-term research is crucial to support the direct seeding technique recommendation.

Our results for the Shannon index (H') ranged from 2.01 to 2.42 nat ind^{-1}, with high values for treatment including bacterial consortium (Table 2). Our values were lower compared to reported by Cordeiro et al. (2020), who observed H› values between 3.43 and 3.87 nat ind^{-1}. This discrepancy may be attributed to differences in forest age and the location of the plots within preservation areas or legal reserves. Younger or recently restored forests, such as those in our study, often exhibit lower diversity due to early successional stages and limited species establishment (Suganuma and Durigan, 2015). The same inference can be done when analyzing Pielou's index values, presented a range of 0.28 to 0.35 (Table 2) and decreasing along time, pointed differences in species abundance. The higher evenness in DS+BAC treatment indicates that bacterial consortium can contribute for a more equal distribution among the species. When analyzing Sorensen index, we can also report that treatments using bacterial consortium present more similarity.

Direct seeding is potentially a more cost-effective alternative to conventional tree planting for restoring tropical forest ecosystems (Naruangsri et al., 2023) and it is considered a feasible alternative for large-scale forest restoration, but little is known about the successional trajectory of tropical forests restored through direct seeding (Freitas et al., 2019). According to Ceccon et al. (2016), direct seeding is considered a cheaper and easier alternative technique, since tree seeds are introduced directly on the site rather than transplanting seedlings from nurseries. Although, Grossnickle and Ivetić (2017) in a review reported that current research findings that seedling establishment rates are low (i.e. typically around 20% of seeds planted) due to site conditions, seed predation and vegetation competition, and field performance (i.e. survival and growth) is lower than planted seedlings.

Our results indicated that the use of inoculant bacteria and NPK fertilizer can be beneficial to restoration purposes. The costs vary between the tested techniques, with DS+BAC+NPK treatment having the highest cost (BRL 14,756.64 per hectare) and DS treatment being considered the cheapest (BRL 13,581.72 per hectare), since it uses only seeds, without any additional inputs. DS+NPK and DS+BAC treatments have intermediate costs of BRL 14,116.64 and BRL 14,221.72 per hectare, respectively. Although the comparison between DS+BAC+NPK and DS present a difference of BRL 1,174.92 per hectare, when analyzing the values per seedling (according to density), the opposite happens, since high values were found in the most complete technique (DS+BAC+NPK). Defining costs for restoration projects is difficult, since prices vary from region and time according to the market, as well as used species. Brancalion et al. (2019) reported that planting costs in Brazil is approximately US$ 2000 (adopting 1 USD = 3.87 BRL) per hectare, were within the range of those from mostly single project studies in other Latin American, African, and Asian countries (most range from US$1000-3000 per hectare). Raupp et al. (2020) evaluating costs for direct seeding, considering a 1-y-old direct seeded restoration site with 5000 and 2500 seedlings per hectare, reported values of US$ 797 (low success) to US$ 1656 (high success), with costs varying with the success of seedling establishment (1 US$ = 3.93 BRL).

When we compare our costs and the restoration results with the conventional seedling planting (usually each seedling costs BRL 25.00 and 1667 seedlings are planted per hectare), our techniques present significant savings as promoting satisfactory results. In addition, direct seeding offers several advantages, e.g. the logistics of seed planting are considerably simpler and less labor-intensive compared to seedling planting, which requires pit preparation and seedling transport. The combination of NPK fertilizer and bacteria consortium promotes more vigorous plant growth, resulting in a higher density of seedlings per hectare and greater height growth, increasing the success rates and accelerating the development of the restoration areas. Moreover, the use of this technique (DS+BAC+NPK) can improve soil health, promoting a healthy microbiota that can enhance plant growth and resistance to diseases and environmental stresses (Pandey et al., 2012).

CONCLUSIONS

All direct seeding techniques demonstrated satisfactory results in plant height, density, frequency, species richness, evenness, and community similarity, with the DS+BAC+NPK method outperforming in most evaluated variables. Although DS+BAC+NPK is approximately 8% more costly than traditional direct seeding, its advantages for soil health and the promotion of a more abundant and higher-quality plant community composition justify the investment.

Long-term, diverse research remains essential to fully understand the benefits of supplementary inputs on the long-term success of forest restoration in various ecological contexts.

ACKNOWLEDGMENTS

The authors extend their gratitude to Paulo Daniel Guimarães Guerra de Rezende from SEAP Eucalipto Tratado for funding the research and providing access to the study area. They also thank Dr. Diego Bonaldo Genuário from Biodiversitá for developing the bacterial consortium tailored specifically for native species seeds, and Dr. Danilo Roberti Alves de Almeida from Universidade do Carbono for his collaboration in the experimental design. This study was financed partly by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant number 305377/2021-3.

AUTHORSHIP CONTRIBUTION

Project Idea: PARS; GMC

Funding: PARS;

Database: PARS; GMC

Processing: AR; MS

Analysis: AR; MS; ACF

Writing: PARS; AR; KPP; ACF

Review: PARS; AR; GMC; MS; KPP; ACF

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