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Thales Castilhos de Freitas

**Does diversity beget diversity? The influence of variation in nucleation techniques on
species and functional composition in early Atlantic Forest restoration**

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Thales Castilhos de Freitas

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RESUMO

Restaurar ecossistemas para mitigar os efeitos da degradação ambiental é um desafio urgente, especialmente em áreas onde a perda de biodiversidade e o declínio dos habitats naturais estão em aceleração. Entre os mecanismos para se restaurar os ecossistemas, estão as técnicas de nucleação que são estratégias que visam à restauração de áreas degradadas através da formação de núcleos de vegetação, que servem como pontos focais para a regeneração natural de outras espécies de plantas. A fim de conhecer as técnicas de nucleação e seus padrões espaciais e metodológicos utilizado no Neotrópico, além de testar a eficiência da nucleação em campo na Mata Atlântica, a presente tese foi organizada em dois capítulos: O primeiro capítulo consistiu na revisão sistemática de artigos científicos que aplicaram técnicas de nucleação em ecossistemas terrestres neotropicais. Através da revisão de 58 estudos, identificamos 12 técnicas, sendo os mais frequentes, poleiros artificiais, transposição de solo, ilhotas de mudas e galharias. Observamos que os padrões espaciais e metodológicos variam conforme o *stakeholder* envolvido (pesquisadores, setor privado e agências governamentais) e o histórico de uso da terra. Pesquisadores frequentemente aplicaram técnicas combinadas, enquanto o uso de técnicas isoladas prevaleceu entre os outros *stakeholders*. Áreas como pastagens abandonadas, áreas de mineração e protegidas apresentaram maior variedade de técnicas. Constatamos que o avanço no uso de combinações e a padronização de práticas são fundamentais para ampliar a eficácia da nucleação na restauração ecológica. No segundo capítulo, conduzimos um experimento para avaliar a diversidade de espécies e funcional utilizando técnicas de nucleação aplicadas em pasto abandonado na Mata Atlântica. Utilizamos nove técnicas de nucleação, partindo de um gradiente de diversidade de técnicas, compondo tratamentos com uma técnica de nucleação, três técnicas do mesmo grupo, três técnicas de grupos diferentes (uma de cada grupo) e nove técnicas (diversidade máxima). Após 15 meses, observamos que a nucleação aumentou significativamente a diversidade de espécies e funcionalidade ecológica, com destaque para técnicas que introduzem sementes, como ninho de sementes e transposição de solo. A combinação de técnicas promoveu maior diversidade de espécies, embora a funcionalidade ainda não tenha alcançado o mesmo padrão. Identificamos espécies-chave capazes de superar os filtros ambientais da área degradada, com potencial para aplicação em projetos de restauração em contextos similares. Esses resultados reforçam o potencial da nucleação como uma estratégia eficiente e escalável para restaurar paisagens degradadas.

Palavras-chave: Área degradada; Diversidade; Ecossistema florestal; Restauração ecológica.

ABSTRACT

Restoring ecosystems to mitigate the effects of environmental degradation is an urgent challenge, particularly in areas where biodiversity loss and the decline of natural habitats are accelerating. Among the mechanisms for ecosystem restoration, nucleation techniques stand out as strategies aimed at restoring degraded areas by creating vegetation nuclei, patches of vegetation that serve as focal points for the natural regeneration of other plant species. This thesis is organized into two chapters to examine the spatial and methodological patterns of nucleation techniques in the Neotropics and evaluate their field effectiveness in the Atlantic Forest. The first chapter provides a systematic review of scientific articles that have nucleation techniques in Neotropics terrestrial ecosystems. From the review of 58 studies, we identified 12 techniques, with artificial perches, topsoil transfer, seedling islets, and brushwood being the most frequent. Spatial and methodological patterns varied depending on the stakeholders involved (researchers, private sector, and government agencies) and the land-use history. Researchers frequently applied combined techniques, whereas the use of isolated techniques prevailed among other stakeholders. Greater diversity of techniques was observed in abandoned pastures, mining areas, and protected areas. Advancing the use of combined techniques and standardizing practices is crucial for enhancing the effectiveness of nucleation in ecological restoration. The second chapter focuses on a field experiment evaluating species and functional diversity using nucleation techniques applied to an abandoned pasture in the Atlantic Forest. Nine nucleation techniques were tested, following a gradient of diversity, with treatments including one technique, three techniques from the same group, three techniques from different groups (one from each group), and nine techniques (maximum diversity). After 15 months, we found that nucleation significantly increased species diversity and ecological functionality, with techniques involving seed inputs, such as seed nests and topsoil transfer, showing the highest plant recruitment. Combining techniques promoted greater species diversity, although functional diversity did not yet follow the same pattern. We identified key species capable of overcoming environmental filters in the degraded area, with potential for use in restoration projects in contexts with similar land-use histories. These

findings highlight the potential of nucleation as an efficient and scalable strategy for restoring degraded landscapes.

Keywords: Degraded area; Diversity; Ecological restoration; Forest ecosystem.

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1 General Introduction

Overexploitation, habitat suppression, biological contamination, and climate change are accelerating species extinction processes and undermining critical ecosystem services, such as climate regulation, nutrient cycling, and pollination, that are essential for human well-being (Ceballos et al., 2017). These threats have both immediate and long-term consequences, leading to cascading ecological disruptions that compromise ecosystem resilience and the provision of essential services. Given the urgency of mitigating these effects, ecological restoration emerges as a key strategy to promote habitat recovery and biodiversity conservation. However, effective restoration requires coordinated action among multiple sectors such as governments, scientific communities, private enterprises, and civil society, to maximize success and ensure long-lasting impacts (Suding, 2011). In response to this global challenge, and in alignment with Sustainable Development Goal (SDG) 15, the United Nations declared 2021–2030 as the Decade on Ecosystem Restoration, fostering collective efforts to rehabilitate degraded ecosystems, mitigate climate change, enhance food and water security, and curb biodiversity loss (Aronson et al., 2020).

Ecological restoration serves as a critical tool to reestablish ecosystem processes, such as interspecific interactions and ecological succession, through targeted human intervention (Brancalion et al., 2015). Within this framework, nucleation techniques offer an innovative and ecologically grounded approach to restoring degraded areas by forming vegetation nuclei that catalyzes natural regeneration. Drawing inspiration from natural successional processes, these techniques create microhabitats that facilitate the colonization of plant species and foster ecological interactions (Reis et al., 2014). By addressing ecological barriers, such as seed dispersal limitations and poor soil conditions, nucleation techniques enhance the structural, functional, and species diversity of ecosystems (Reis et al., 2014; Rojas-Botero et al., 2020). For instance, nucleation techniques can provide shade, enhance soil organic matter, and attract seed dispersers such as birds and mammals, thereby facilitating the establishment of woody species and other plants with limited dispersal capacity. Facilitation plays a central role in this process, as pioneer species and functionally diverse plants improve environmental conditions for subsequent species by alleviating stresses such as intense solar radiation, wind exposure, and nutrient scarcity (Corbin and Holl, 2012). Additionally, a greater diversity of plants increases the likelihood of including facilitative species, such as nitrogen-fixing plants, which enhance soil fertility and create conditions favorable for the establishment of other species,

ultimately boosting species richness and advancing ecosystem restoration. Techniques like artificial perches, soil transposition, and the creation of vegetation islands exemplify the practical implementation of nucleation, demonstrating its capacity to accelerate ecological succession and promote the establishment of self-sustaining ecosystems (Reis et al., 2014).

The ideal approach to ecological restoration is the use of a diverse set of restoration techniques, as, in addition to associated costs, the chosen technique and species selection can lead to different trajectories in the restoration process. This factor is crucial for achieving a balance between cost-effectiveness and the restoration of the desired ecosystem (Bechara et al., 2021; Cruz et al., 2020). This heterogeneity enhances restoration by increasing the likelihood of the arrival of plant species with different and complementary functional traits, thereby influencing the restoration process. This approach is grounded in the ecological theory of niche complementarity (Tilman, 1999), which posits that in a heterogeneous system, species exhibit distinct traits that determine their success in these environments. This diversity reduces interspecific competition by increasing niche partitioning, allowing each species to coexist and utilize available resources more efficiently. Consequently, this process enhances ecosystem functioning, reflected in greater biomass productivity, more robust trophic interactions, improved soil conditions, and ultimately, increased success in ecological restoration (Naeem et al., 1999). In more diverse communities, the probability of species complementing each other is greater, and this can be achieved through the use of different restoration techniques.

Among the priority areas for restoration is the Atlantic Forest biome, which began to be exploited unsustainably in the early 15th century and has suffered drastically to this day from the loss of natural areas, mainly due to deforestation due to conversion into areas for agriculture, crops and real estate speculation. The Atlantic Forest was selected by the Convention on Biological Diversity (CBD) as one of the global hotspots (Myers et al., 2000) due to its great biodiversity, endemism, and high risk of degradation (Eisenlohr et al., 2015). Currently, the Atlantic Forest retains approximately 24% of its original forest cover when considering fragments of all sizes. However, this percentage drops to 12.4% when examining fragments larger than three hectares (SOS Mata Atlântica, 2024) and decreases drastically to just 8.5% for fragments exceeding 100 hectares (Projeto MapBiomias, 2024). Most forest remnants in this biome consist of small, highly fragmented patches with low connectivity and are predominantly composed of secondary or non-forest vegetation. Research suggests that conserving these forests requires maintaining at least 30% forest cover within the landscape,

regardless of the conservation status of these areas (Metzger, 2010; SOS Mata Atlântica, 2024). Consequently, restoration initiatives are becoming increasingly common. However, a significant challenge to restoring the Atlantic Forest is the prevalence of exotic pastures, primarily composed of species from the *Urochloa* genus (commonly known as braquiária) and the *Melinis* genus (fat grass). These invasive grasses form dense vegetation mats that can delay or even prevent natural regeneration and hinder the establishment of species used in restoration projects (Sartorelli and Campos-Filho, 2017).

Conventional forest restoration techniques are costly and labor-intensive, and in many cases are inefficient, as they depend on biotic and abiotic factors for their success, in addition to constant maintenance. Thus, the search for and validation of alternative, efficient and lower-cost techniques can create more favorable conditions for the success of ecological restoration, especially when considering the various trophic aspects and ecological interactions. In this scenario, the study of functional diversity and functional traits of plants is of relevant importance, allowing us to understand how different species influence these key processes in the initial stages of restoration and how different nucleation strategies can shape ecological restoration in abandoned pasture areas.

2. Chapter 1: Assessing nucleation strategies and spatial dynamics in the restoration of Neotropical terrestrial ecosystems: A comprehensive review

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2.1 Abstract

Neotropical ecosystems have been significantly impacted by agricultural activities and urbanization, causing biodiversity and ecosystem services losses, and requiring extensive restoration efforts. Thus, nucleation strategies appear as a cost-effective restoration method, which promotes ecological succession by establishing vegetation patches that attract fauna and facilitate natural regeneration. Here, we systematically reviewed nucleation techniques applied for Neotropical ecosystems restoration. We assessed the focus and spatial distribution of nucleation in response to stakeholder preferences and land-use types. By examining 58 studies, we identified key aspects of the use of nucleation techniques, emphasizing the need for standardized protocols to optimize nucleation strategies for ecosystem recovery. Overall, 12 nucleation techniques were used in Neotropical ecosystem restoration, with artificial perches, topsoil transfer, seedling islets and brushwood being the most frequent. Stakeholders (i.e., researchers, private sector, and governmental agencies) applied various techniques, with researchers more frequently using multiple and combined techniques. Moreover, we observed that stakeholders and land use affect the spatial patterns of nucleation, whereas their focus only responds to land-use types. Indeed, fauna-related techniques were common across all land uses, and the greatest variety of techniques were used in abandoned pastures, former mining sites and protected areas. Although less frequent, using nucleation techniques in combination can contribute to restoration success by addressing multiple objectives. Research-driven innovation is advancing this field, but single techniques dominate. Advancing the use of combined techniques and standardizing such practices is essential to scale up and optimize nucleation strategies during restoration efforts.

Keywords: Nucleation; Land use; Nucleation focus; Restoration techniques; Stakeholders.

2.2 Implications for practice

- Most restoration projects rely on single nucleation techniques, overlooking the potential of nucleation strategies to recover multiple ecosystem properties.
- Combining nucleation techniques with different focuses offers a more effective restoration strategy.
- Previous land uses determine the choice of nucleation techniques, suggesting that restoration initiatives using nucleation should account for land-use history to foster ecological restoration.
- Researchers are the most frequent stakeholders involved with nucleation, yet government agencies and the private sector can contribute to scaling these approaches to broader restoration projects and goals.
- The lack of standardization in the use of nucleation techniques hinders scalability, underscoring the need for clearer guidelines developed in collaboration with the different stakeholders to optimize restoration efforts.

2.3 Introduction

Over the past decades, Neotropical terrestrial ecosystems underwent major land use changes, mostly due to agriculture expansion and urbanization (Laurance et al. 2014; Brancalion et al. 2019). Studies in tropical forests showed that deforestation and conversion into pastureland result in the fragmentation and significant degradation of such ecosystems (Barlow et al. 2016; Magioli et al. 2021). Indeed, the intensification of crop plantation and cattle breeding has led to biodiversity losses and to altered local and global climate patterns in Neotropical areas (Kehoe et al. 2017; Garret et al. 2018). Areas converted into pasture often experience soil erosion, nutrient depletion, and reduced capacity to support fauna and flora diversity (Barlow et al. 2016; Van der Sande et al. 2023). When abandoned, these pastures are typically characterized by low soil fertility and dominance by invasive plant species, which inhibit natural regeneration and compromise restoration efforts (Elgar et al. 2014; Holmes and Matlack 2019; Rojas-Botero et al. 2020). The impacts of such environmental degradation extend to the quality of life of local communities, contributing to a higher incidence of poverty in regions affected by soil degradation (UNCCD, 2017). Faced with the changes caused by environmental degradation, the United Nations (UN) established the Decade of Ecosystem Restoration (2021–2030) (UNEP and FAO, 2020) to intensify efforts in combating the climate crisis, halting biodiversity loss, improving food sovereignty and water resources, and promoting ecological restoration through international commitments and targets (Aronson et al. 2020).

To achieve local as well as global restoration goals, it is necessary to develop and validate ecological restoration strategies and techniques that are both efficient and economically viable (Matzek et al. 2017; Bechara et al. 2021; Toro et al. 2024). Among the strategies to promote ecological restoration, nucleation techniques stand out as an approach aimed at creating conditions for ecological succession to occur as naturally as possible. This is achieved through the establishment of vegetation patches, or nuclei, which mimic natural colonization processes (Yarranton and Morrison, 1974). In practice, nucleation facilitates the establishment of pioneer plant communities, which modify the environment and create conditions for the recruitment of additional species. This process stimulates ecological interactions and dynamics across the food chain, as the expanding nuclei attracts key restoration agents such as birds, microorganisms, insects, seeds, and plants, gradually contributing to the recovery of degraded areas (Corbin and Holl, 2012; Bechara et al., 2016).

Indeed, such nuclei play a critical role in fragmented landscapes by serving as ecological stepping stones, facilitating connectivity and gene flow between degraded and non-degraded areas and supporting the maintenance of ecological processes across the landscape (Reis et al., 2010).

One of the main advantages of nucleation is the need for less financial and labor resources compared to traditional sapling planting. Indeed, the nucleation techniques can be used with available natural materials, such as branches, pruning residues, seeds, or by densely planting seedlings, all installed in nuclei of different sizes, depending on the area and resources available (Bechara et al. 2021). These techniques facilitate ecological restoration across multiple trophic levels, targeting soil, vegetation, and fauna. For instance, soil improvement can be achieved through topsoil and litter transfer techniques, vegetation can increase through direct seeding and seedling islets techniques, and fauna can be attracted through the use of artificial perches and brushwood techniques (Fig. 1; Rojas-Botero et al., 2020; Bechara et al., 2021). Monitoring trophic levels in restoration ecology is essential for understanding ecosystem recovery, as it incorporates trophic interactions beyond vegetation, providing valuable insights to assess restoration success and develop effective management strategies (Fraser et al., 2015; Loch et al., 2020). Furthermore, recent evidence showed that the combination of nucleation techniques such as topsoil transposition and bird perches produce better restoration outcomes (Bahia et al. 2023).

The use of nucleation in ecological restoration projects involves a range of techniques, such as varying nuclei sizes, densities (e.g., density nuclei per hectare), and spacing (e.g., distance between nuclei), which are tailored to local conditions and project objectives. These spatial patterns can significantly affect key ecological processes, such as propagule arrival, soil conditions, and animal use of nuclei for foraging, resting, or seed deposition, thereby influencing restoration success (Sasal and Morales, 2013; Bahia et al., 2023). Additionally, assessing the land use history is crucial, as it provides valuable insights into the extent of degradation, guiding the selection of appropriate restoration strategies. By understanding past impacts and current constraints, stakeholders can better tailor nucleation approaches to align with ecological objectives, socioeconomic needs, and available resources (Hernandez et al., 2024; Bechara et al., 2016; Holl et al., 2020; Bahia et al., 2023).

More recently, a review and meta-analysis study was able to assess how different nucleation techniques affect the plant survival and establishment as well as diversity and abundance in restoration projects (Bahia et al. 2023). The same study also evaluated the

performance of nucleation techniques according to vegetation and previous land use types (Bahia et al. 2023). Still, there is a need for further research to guide the development of more consistent and effective protocols for the use of nucleation techniques in restoration projects. Hence, conducting a systematic review on nucleation strategies during the restoration of Neotropical terrestrial ecosystems can address this research gap, potentially contributing to the scalability of restoration efforts. By systematically reviewing the scientific literature, we expect to identify patterns, knowledge gaps, and key determinants of the use of nucleation in the restoration of terrestrial ecosystems in the Neotropics, such as technique selection, site conditions, and stakeholder interests.

Thus, in this paper, we systematically reviewed the use of nucleation techniques, their size and spatial distribution according to land use and stakeholder preferences for the restoration of terrestrial ecosystems in the Neotropics. Hence, we asked the following questions: (1) What are the main nucleation techniques used during the restoration of Neotropical terrestrial ecosystems? Is there a dominant technique? (2) Are such techniques used separately or in combination? Which combinations are the most common? (3) Do stakeholder groups and previous land use types affect the choice of the nucleation technique focus used for the restoration of terrestrial ecosystems in the Neotropics? (4) What is the density per hectare, average size and distance between the nuclei applied for the restoration of such areas? Do stakeholders, previous land use, and the focus of the nucleation technique influence these characteristics? (5) Are the use of alone-to-combined, as well as single-to-multiple techniques, influenced by stakeholders and land-use types?

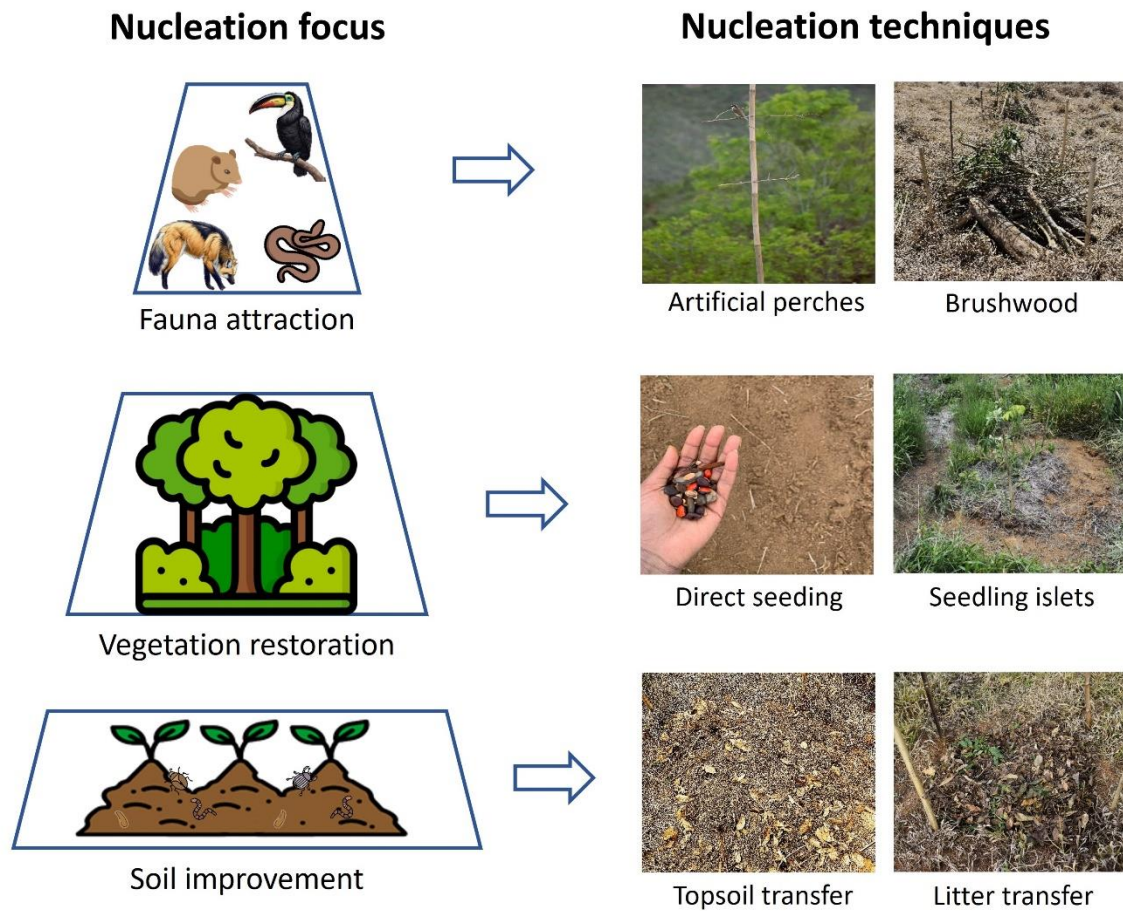


Figure 1. Schematic illustration depicting nucleation as a versatile method for ecological restoration. It allows targeted interventions across different trophic levels via the use of techniques aimed at e.g. improving soil conditions (topsoil and litter transfer), restoring vegetation (direct seeding and seedling islets), or attracting fauna (artificial perches and brushwood). Trapezoid shapes indicate the trophic levels impacted by these nucleation techniques. Note that soil, vegetation, and fauna icons are for illustrative purposes only. Photos of all nucleation techniques are provided by the authors.

2.4 Methods

2.4.1 Systematic review

To identify the differences in the spatial distribution patterns of nucleation as well as the influence of land use types and stakeholder groups on the techniques used in restoration projects in Neotropical areas, we conducted a comprehensive search for relevant literature by querying keywords in both the *Web of Science Core Collection* and the *Scopus* databases.

This search was carried out between November 2023 and March 2024, covering the period between 2000 and 2024. The literature search and review were conducted following procedures and recommendations provided by Haddaway et al. (2018) and Collaboration for Environmental Evidence (2013). Our review study's rationale, objectives, and planned methods are shown in Fig. S1. Afterwards, the defined search terms were combined in different ways using the Boolean operator 'OR'. We first tested the performance of our search terms in Web of Science and then replicated the search in the Scopus database. Our search used the following terms: *Applied nucleation* OR *Vegetation nuclei* OR *Nucleation* OR *Island** OR *Restoration* combining with *Technique** OR *Facilitation* OR *Regeneration* OR *Seedling* OR *Establishment* OR *Perch** OR *Brushwood* OR *Transposition*. The asterisk (*) included all possible variations of a word root, ensuring that our search captured all relevant studies related to different keyword forms. Considering that our review study assesses the use of nucleation strategies for the restoration of Neotropical terrestrial ecosystems, the search was conducted in English, Spanish, and Portuguese.

From the *Web of Science*, our search retrieved 3,043 papers of which 353 were related to ecological restoration. From the Scopus database, we initially obtained 14,869 papers of which 7,447 concerned ecological restoration. Thus, our initial dataset comprised 7,710 publications. Because we were interested in assessing the design and implementation characteristics of nucleation strategies and to avoid pseudo replication, we excluded publications that were conducted in the same study area, thus selecting the first published article as the representative of such a location.

2.4.2 Screening, inclusion criteria, and assessment of the selected studies

Our review study considered different inclusion criteria. Our dataset comprised studies published in the last 23 years and included scientific papers, dissertation, thesis and two reviews on nucleation (i.e., Guidetti et al., 2016; Bahia et al., 2023) (Table S1). Such a time span also considers the advances in nucleation as a restoration strategy and its use for the restoration of Neotropical terrestrial ecosystems during the past decades (Holl et al., 2020; Bahia et al., 2023). After the literature search on the Web of Science and Scopus databases, the publications obtained were manually screened. Search results were sorted for their specific topics, keywords, and potential duplicates. Afterwards, the articles retrieved were screened by

title and abstract. Furthermore, conceptual frameworks, scientific notes, book chapters, and purely qualitative studies were excluded (Fig. S1).

We only selected studies that met the following criteria: (i) conducted in terrestrial systems in the Neotropics, (ii) testing nucleation techniques, (iii) under field conditions, (iv) stating the type of techniques tested, and (v) providing quantitative surveys. Studies not meeting one or more of these selection criteria were excluded from our review. Further selection was performed considering the abstract and methods section of the publications. After running the systematic review parameters and analyzing the ca. 8,000 results stepwise from the title and abstract to full-text screening, 58 studies fitted the predefined review parameters (Fig. S1; Table S1). Within those studies, three main stakeholder groups were observed applying nucleation techniques for the restoration of terrestrial ecosystems in the Neotropics; i.e., scientific researchers and academics (R), as well as actors of the private sector (P) and government agencies (G). The different stakeholders were determined according to their correspondence addresses and the area where the work took place (e.g., public or private lands, nature reserves, etc.). This dataset enabled the examination of our five research questions.

2.4.3 Data extraction and statistical analyses

After identifying the suitable articles, we extracted information on stakeholder identities, the previous land use of the areas under nucleation, the type and number of nucleation techniques used, and the year of each publication. Details on site conditions (related to the previous land use types) were obtained from the abstract or the methods section of the publications, i.e., (i) abandoned pastures, (ii) forestry, (iii) mining, (iv) natural regeneration, (v) protected areas, (vi) urban areas, and (vii) landslides. Afterwards, we retrieved data on the use of multiple vs. single nucleation techniques and whether those techniques were applied alone or in combination. Thus, we obtained the ratio of multiple-to-single as well as the ratio of combined-to-alone techniques being used to assess the development and specialization of nucleation. This was done by assigning ‘one’ to studies applying two nucleation techniques or more and ‘zero’ to studies using single techniques. The ratio of alone-to-combined techniques followed the same approach. Therefore, such data had a binomial distribution. We categorized the nucleation techniques identified in each publication into four groups based on their primary ecological objectives and the trophic

levels at which their effects can be observed (i.e., soil, flora, fauna, and multiple; Fig. 1). The first group, soil restoration, focuses on microorganisms and decomposers that play a critical role in improving soil conditions, thus enabling better vegetation establishment. Although microorganisms do not clearly fit into a specific trophic level, they are included as a distinct group with specific restoration function. The flora restoration group focuses on primary producers with the main goal of improving vegetation, particularly via planting saplings and sowing seeds. Finally, the fauna group consists of fostering the attraction of consumers, which facilitate gene flow and ecological interactions by employing techniques like artificial perches and brushwood (Fig. 1). This allowed us to assess the variation in the aims of nucleation strategies during the restoration of terrestrial ecosystems in the Neotropics.

Subsequently, we retrieved information on the size of the nucleation plots (m^2 ; hereafter nuclei size), the density of nuclei per hectare ($N.ha^{-1}$), the distance or spacing between nuclei (m) and the total size of the area under nucleation (nuclei size plus spacing; measured in ha). Information on nuclei size was extracted from the studies, although two studies did not report that for some nucleation techniques. For the density of nuclei per ha, when the studies were carried out on up to 1 ha, this information was extracted directly. However, when project areas were larger than 1 ha, we divided the number of plots by the total size of the nucleation project area. If the study did not provide the total size of the area under nucleation (i.e., $N = 14$ publications), but included the distance between the nuclei and the nuclei size, the density of nuclei per ha was calculated based on such information. In this case, the density of nuclei per ha was determined by dividing 10,000 by the nuclei size (in m^2) and the surrounding spacing (in m^2), using the formula: $Area = 10,000 / (S + E)^2$; where S represents the nuclei size and E the spacing between the plots. The spacing between the nuclei and the size of the total area under nucleation could only be used in the calculations when this information was provided in the studies.

We assessed the differences in nucleation techniques, their focuses among stakeholder groups, and their relation to previous land use types, using Fisher's Exact Test with 10,000 Monte Carlo simulations (two-tailed test), implemented via the stats package in R (R Core Team 2023). This analysis was applied to verify how the different nucleation techniques identified in this review and their focuses are affected by stakeholder preferences and land use types. Afterwards, we used linear mixed-effects models (LMM) to test the effects of the stakeholder groups, nucleation technique focus (i.e., fauna, flora, soil, or multiple), and their interaction on the spatial and quantitative parameters of the nucleation (i.e., nuclei size, nuclei

density per hectare, spacing between nuclei, and the total size of the area under nucleation). These models included time (i.e., publication year) and previous land use types as random intercepts. Similar models were applied to test the individual and the interaction effects of previous land use types and nucleation technique focus on the same spatial and quantitative parameters mentioned above. For such models, random intercepts were stakeholders and time. All response variables were log-transformed prior to these analyses, to fulfill the assumptions of normality of residuals and homogeneity of variances.

Finally, a generalized linear model (GLM) with a binomial error structure was calculated to test for the individual effects of stakeholders and previous land use types on the ratios of multiple-to-single and combined-to-alone nucleation techniques. Because not all stakeholder groups worked in areas affected by all land-use types, we could not test their interaction effects on the abovementioned ratios. Significant effects were determined through a Wald Test (type 3) using the function ANOVA of the car package (version 3) in R (Fox and Weisberg 2019). All models were calculated with the R-package *lme4* version 1.1.34 (Bates et al., 2015). Statistical analyses were performed in R version 4.3.1 (R Core Team 2023). Model performance was assessed graphically and with the R-package *DHARMa* (Hartig, 2020).

2.5 Results

2.5.1 Nucleation techniques and their focus according to stakeholder groups and previous land uses

We found 12 nucleation techniques being used as restoration strategies in 58 studies in the Neotropical region. The most frequent nucleation techniques used were artificial perches (23% of studies), topsoil transfer (18%), seedling islets (17%), brushwood (10%), and combined techniques (9%) (Fig. S2). Most articles use the techniques separately (i.e., ca. 85% of all studies). Still, ten studies applied different nucleation techniques in combination (Fig. S3). These combinations varied between two or three techniques, highlighting the combination of brushwood and artificial perches. When we look at the number of techniques used together, seven of the 12 techniques mentioned were combined in some way. Regarding studies that employed either a single or multiple nucleation techniques, 61% used only one type of technique. In turn, those that applied multiple techniques incorporated between two and seven different nucleation techniques.

Our results indicated that researchers (R) were involved in the application of all nucleation techniques and developed projects in cooperation with all other stakeholder groups. Also, when researchers were involved, all the possible nucleation focus groups (i.e., soil, flora, fauna, and multiple) were covered. Meanwhile, the private sector (P) collaborated mostly with researchers, using 10 of the 12 nucleation techniques identified. Researchers primarily utilized artificial perches and seedling islets on their own, while in partnership with the private sector, they focused on artificial perches and topsoil transfer. When we compared the focus of the nucleation techniques used by the stakeholder groups, we observed similar proportions among different focuses (Fig. 2). No publications were found in which the private and government sectors worked alone, only in cooperation with researchers.

Although not detecting any significant differences when comparing the proportion of nucleation technique focus (i.e., soil, flora, fauna, and multiple focuses) across the different stakeholder groups (i.e., R, RP, RG, PG, and RPG; Fisher's Exact Test with 10,000 Monte Carlo simulations: $p = 0.8$; 95% CI: 0.851–0.865; Fig. 2A), we observed that fauna- and flora-related techniques are well-represented across the majority of the stakeholders, with a particularly high presence when all stakeholders worked in collaboration (RPG). Moreover, soil-related techniques were the primary focus when stakeholders of the private sector collaborated with the government (PG). This is so, because only one publication reported on the collaboration between the private sector and government (Fig. 2A). Finally, techniques with multiple focuses (indicating the use of nucleation techniques with effects on distinct trophic levels; see Fig. 1) are, to some extent, applied across most stakeholder groups but have a smaller contribution overall, except in RPG, where their use is more moderate (i.e., < 25%; Fig. 2A).

The distribution of nucleation technique focuses varied significantly with previous land-use types (i.e., abandoned pastures, forestry, landslides, mining, natural regeneration, protected areas, and urban areas; Fisher's Exact Test with 10,000 Monte Carlo simulations: $p \leq 0.05$; 95% CI: 0.0098–0.0140; Fig. 2B). Our findings showed that fauna-related techniques are employed in all land-use types, particularly dominating in areas affected by landslides and urban settings, while flora-related techniques are widespread, especially in abandoned pastures and areas formerly used for forestry activities. In turn, soil-related techniques are most prevalent in mining, where they account for half of the cases. Albeit in a lower frequency, such techniques are also used in areas under all other land use types, except

landslides (Fig. 2B). Interestingly, multiple-focused techniques were observed in several land-use types, with highest application in natural regeneration and urban areas (Fig. 2B).

Overall, these findings show that previous land-use types (Fisher's Exact Test with 10,000 Monte Carlo simulations: $p \leq 0.05$; 95% CI: 0.0098–0.0140) but not stakeholder groups (Fisher's Exact Test with 10,000 Monte Carlo simulations: $p = 0.8$; 95% CI: 0.851–0.865) significantly influence the choice of nucleation techniques and their respective focus (Fig. 2). Whereas stakeholders from the private sector cooperating with the government (PG) focus mostly on soil-related techniques, all stakeholders working together (RPG) tend to balance a mix of fauna- and flora-related techniques (Fig. 2A). Furthermore, we observed that restoration projects aiming at recovering abandoned pastures exhibited the greatest diversity of nucleation techniques being applied ($N = 11$), followed by projects implemented in areas under former forestry use ($N = 10$) and in protected areas ($N = 8$). Studies developed in former mining areas as well as in urban settings and in areas under natural regeneration utilized six or fewer nucleation techniques each (Fig. S4). Notably, nucleation in landslide areas was represented by only one study, which utilized artificial perches. Flora-related nucleation techniques were most frequently applied in areas formerly under forestry and pasture uses, with all techniques being utilized in both land use types (Fig. 2B). Restoration projects in protected areas employed all types of soil-related nucleation techniques, while both pasture and forestry land uses applied all flora-related techniques (Fig. 2B).

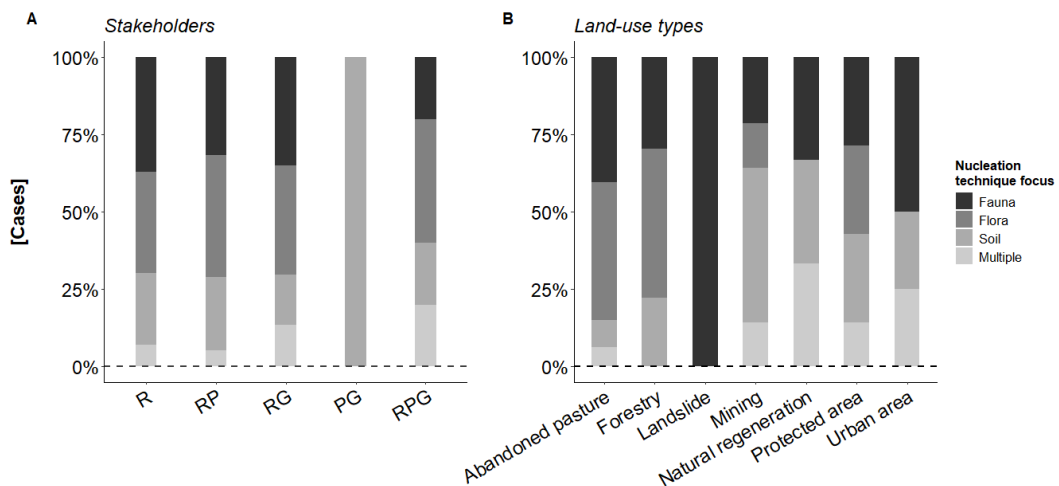


Figure 2. Frequency of studies applying nucleation techniques with their focuses separated according to stakeholder groups (R: $N = 43$; RP: $N = 38$; RG: $N = 37$; PG: $N = 1$ and RPG: $N = 5$) and previous land use types (Abandoned pasture: $N = 47$; Forestry: $N = 27$; Landslide: $N = 5$).

= 1; Mining: N = 14; Natural regeneration: N = 3; Protected area: N = 28 and Urban area: N = 4).

2.5.2 Quantitative parameters and spatial distribution of the nuclei

The nuclei had an average size of 67.58 m² ($\pm$ 227.14 m²), with sizes varying from 0.16 m² to 1225 m². The average density of nuclei per hectare was 121.10 N.ha⁻¹ ($\pm$ 255.92 N.ha⁻¹), with densities ranging from 0.27 to 1600 nuclei per ha (N.ha⁻¹). The average spacing between the nuclei was 9.17 m $\pm$ 20.21 m, ranging from 0.3 m to 150 m. Only 39 papers provided information on the spacing, making it the most information-deficient variable. The experimental areas using nucleation techniques averaged 13.04 ha ($\pm$ 34.17 ha), with sizes ranging from as small as 0.01 ha to as large as 132 ha.

Stakeholder groups (private sector, academia and government agencies) and previous land use (abandoned pasture, forestry, mining, natural regeneration, protected area, urban area and landslides) had a notable influence on the quantitative and spatial parameters assessed (Fig. 3 and 4). Nuclei size was significantly affected by the interaction between stakeholders and the focus of the nucleation techniques ($X^2 = 18.9$, df = 9, $p = 0.02$) (Fig. 3A), as well as by the interaction between the techniques' focus and previous land use ($X^2 = 23.0$, df = 12, $p = 0.02$) (Fig. 4A). For nuclei density per hectare, the different stakeholders showed a marginal effect ($X^2 = 9.4$, df = 4, $p = 0.05$). Furthermore, the spacing between the nuclei was significantly influenced by both the interaction between technique focus and stakeholders ($X^2 = 43.3$, df = 6, $p \leq 0.001$) (Fig. 3C). and the interaction between technique focus and land use ($X^2 = 57.1$, df = 7, $p \leq 0.001$) (Fig. 4C). Significant variations in spacing were observed across all stakeholders and focus groups, with flora and fauna techniques showing the greatest distances between nuclei. Stakeholders ($X^2 = 22.1$, df = 4, $p \leq 0.001$) and previous land use ($X^2 = 15.8$, df = 5, $p = 0.007$) also had a positive effect on the total size of nucleation projects (Figs. 3D and 4D).

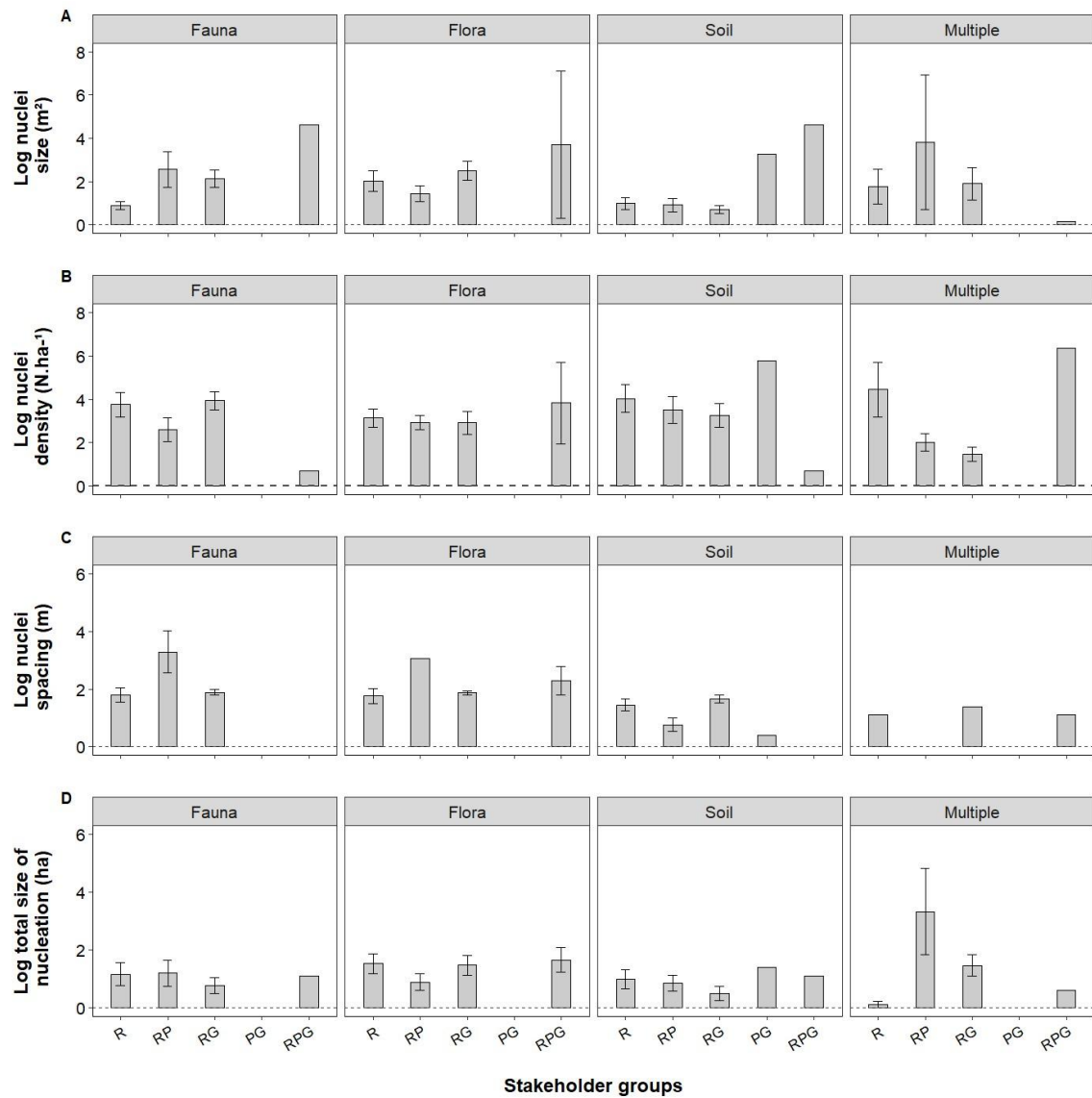


Figure 3. Spatial and quantitative parameters evaluated according to stakeholders and nucleation technique focus. A. Size total area (ha); B. Nuclei density (N.ha⁻¹); C. Spacing between nuclei (m), and D. Total size of nucleation (ha). R. research; RP. research and private sector; RG. research and governmental agency; PG. private sector and governmental agency; RPG. research, private sector and governmental agency. The figure depicts average values as assessed by LMMs. Error bars represent the standard error ($\pm$ SE) of the mean for each group. The figure shows that the nuclei size and spacing between the nuclei were significantly affected by the interaction between stakeholders and the focus of the nucleation techniques. The total size of nucleation was influenced by stakeholders.

Certain nucleation techniques were specific to particular land use types. For example, bromelia islets, plant mats, beans islets, and seed rain were used in pasture and forestry projects, while material from urban prunings and seed banks were applied in urban settings and protected areas, respectively (Fig. S4). Artificial perches were the most widely applied technique, used across all land use types ($N = 7$), followed by topsoil transfer applied in six land use types, combined techniques used in five, and brushwood, direct seeding, and litter transfer, which were applied in four land use types (Fig. S4). Fauna-related techniques were applied across all previous land uses, while the soil-related ones were used in six land use types. Abandoned pastures had the greatest variety of nucleation techniques, with 45% focused on flora, 40% on fauna, 9% on soil, and 6% on multiple aspects, followed by protected areas and forestry. In former mining areas, soil-related techniques were predominant, representing 50% of the techniques used.

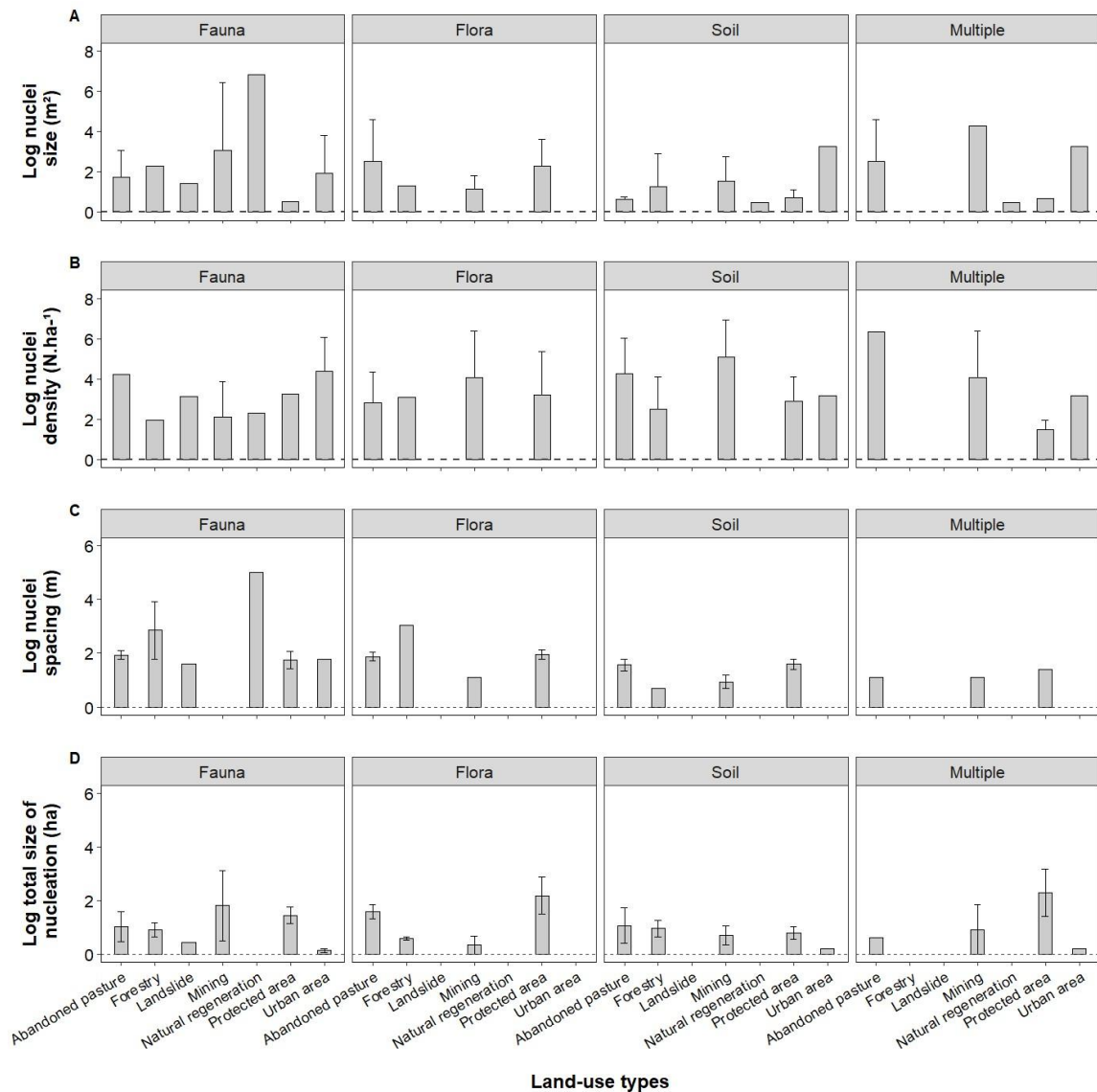


Figure 4. Spatial and quantitative parameters evaluated according to land use types and nucleation technique focus. A. Nuclei size (m²); B. Nuclei density (N.ha⁻¹); C. nuclei spacing (m), and D. Total size of nucleation (ha). The figure depicts averaged values as assessed by LMMs. Error bars represent the standard error ($\pm$ SE) of the mean for each group. The figure shows that the nuclei size and spacing between the nuclei were significantly affected by the interaction between land use and the focus of the nucleation techniques. The total size of nucleation was influenced by land use types.

2.5.3 Ratios of multiple-to-single and combined-to-alone nucleation techniques

Overall, our results indicate no difference in the preference for multiple or single nucleation techniques according to stakeholders and previous land-use types ($X^2 = 6.96$, $df = 4$, $p = 0.1$; $X^2 = 10.61$, $df = 6$, $p = 0.1$; Figs. 5A, B, respectively). Still, we noticed that most

stakeholders (working alone or in collaboration; i.e., R, RP, PG, and RPG) showed a preference for applying single nucleation techniques, while RG used more multiple techniques (60%) (Fig. 5A). Furthermore, we observed that single techniques prevailed in abandoned pastures, landslides and formerly mined areas, whereas forestry favored the use of multiple techniques. Finally, a more balanced use of both approaches was obtained in areas under natural regeneration, protected and urban areas (Fig. 5B).

Contrastingly, the ratio of combined-to-alone application of nucleation techniques was significantly affected by stakeholders ($X^2 = 12.24$, $df = 4$, $p \leq 0.05$; Fig. 5C) and land use types ($X^2 = 14.53$, $df = 6$, $p \leq 0.05$; Fig. 5D), with some groups and environments showing a strong inclination toward combined approaches over the use of techniques alone (Fig. 5). Additionally, we observed that all stakeholders employed combined techniques when collaborating with researchers (Fig. 5C). While the majority of studies where researchers and governmental agencies collaborate (RG) utilized multiple nucleation techniques, these techniques were more often applied individually rather than in combination. Although stakeholders of the private sector and government (PG), showed a preference for nucleation techniques alone, all stakeholders ultimately employed combined techniques. In fact, for stakeholders of the private sector (P), the use of combined techniques represented the smallest proportion among all groups (i.e., 5%) (Fig. 5C). When assessing the use of combined techniques regarding the land use types, we found that areas under natural regeneration and urban areas showed equal use of techniques applied both individually and in combination (Fig. 5D). Only forestry and landslide did not use combined techniques, whereas for other land use types, individual techniques were more commonly applied (Fig. 5D).

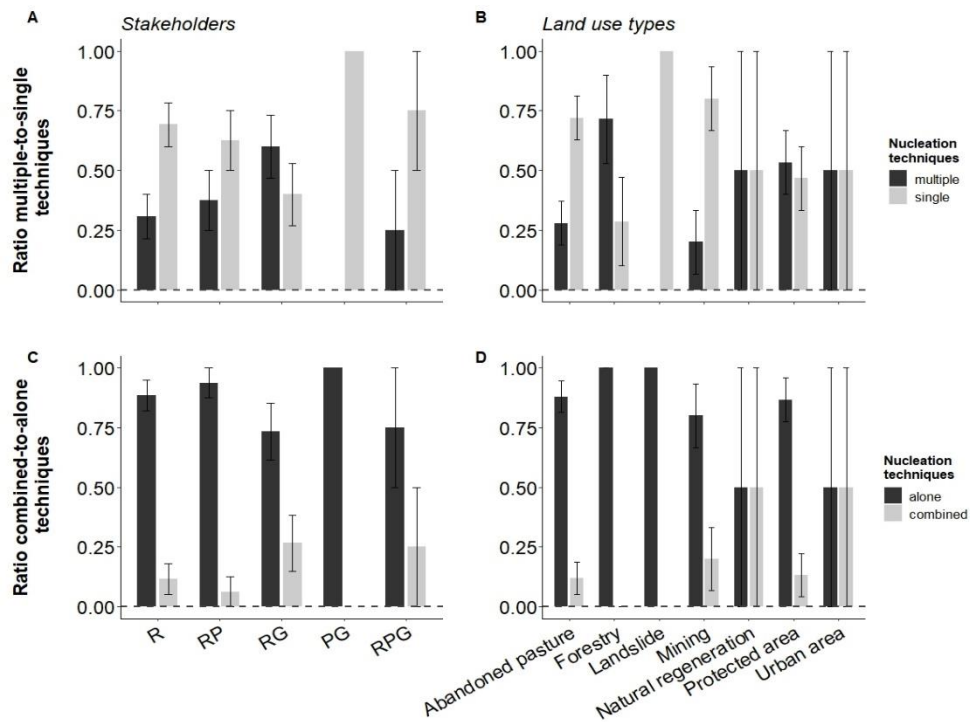


Figure 5. Comparison of the ratios of multiple-to-single (A, B) and combined-to-alone (C, D) nucleation techniques across different stakeholder groups (A, C) and previous land-use types (B, D). The figure depicts averaged values as assessed by GLMs with binomial error structure. Error bars represent the standard error ($\pm$ SE) of the mean for each group. The figure shows that the use of single techniques is favored in most cases. Although multiple approaches were also employed, these techniques are more often used alone.

2.6 Discussion

2.6.1 Nucleation techniques applied alone or in combination

Our review showed that mainly four nucleation techniques are employed for the restoration of terrestrial systems in the Neotropics, i.e. artificial perches, topsoil transfer, seedling islets and brushwood. At least one of these four techniques was utilized in 56 of the 58 publications included in our review. Surprisingly, the application of combined techniques was the fifth most frequent nucleation strategy, however this was only observed in a few publications. Combining complementary techniques can significantly enhance restoration success by addressing multiple objectives simultaneously, such as improving soil conditions through topsoil transfer while facilitating seed dispersal via artificial perches (Bahia et al.

2023). However, to date, the use of single or isolated techniques remains the standard practice in restoration projects applying nucleation techniques (Piaia et al. 2017; Rojas-Botero et al. 2020; Bechara et al. 2021).

Furthermore, the strategic combination of techniques facilitates a more complete and integrated restoration of ecosystems and provides opportunities to experiment with novel approaches and learn from their outcomes. Beyond the recovery of flora and fauna, ecological restoration aims to assist the recovery of various aspects of ecosystems, including their structure, functions, and associated services (Benayas et al., 2009). Indeed, previous evidence shows that the combination of nucleation techniques accelerates ecological succession and prompts initial restoration stages (Marcuzzo et al. 2013; Araujo et al. 2014; Bahia et al. 2023).

We have observed the use of several innovative and creative nucleation techniques based in ecological principles for their selection, such as plant mat (Rojas-Botero et al. 2020), bean and bromelia islets (Bechara et al. 2021), and urban prunings (Marcuzzo et al. 2013). Indeed, nucleation strategies offers the advantage of allowing the development and test of novel techniques or combination of techniques aligning with the established theoretical frameworks of restoration ecology and ecological succession (Van Breugel et al. 2024). While these techniques have only been implemented in a few studies, their innovative approaches can demonstrate significant potential for advancing restoration practices by providing cost-effective and scalable solutions to recover flora and fauna. Despite the emergence of new and combined techniques, their development and advancement are still in the early stages, particularly when it comes to the combination of techniques for improved results.

2.6.2 Stakeholders and previous land use shape nucleation strategies in Neotropical ecosystem restoration

Different stakeholders primarily worked in collaboration, with researchers being the only group to operate independently. Despite this, all major nucleation technique focuses were similarly represented across projects, with no clear preference for specific nucleation technique focus among stakeholders (Fig. 2A). The cooperation between researchers and other stakeholders, along with only one study conducted without the involvement of researchers, underscore the critical role of academia and scientific insight in restoration projects applying nucleation techniques. Research groups typically lead the development and testing of new techniques, and once their effectiveness is proven, these methods are adopted

by private and public entities to address restoration needs. This is evident here, as the four previously mentioned innovative techniques were exclusively applied in projects led by researchers (Marcuzzo et al. 2013; Rojas-Botero et al. 2020; Bechara et al. 2021). The current challenge lies in translating scientific advances into practical, cost-effective restoration strategies capable of promoting the recovery of degraded ecosystems and producing self-sustaining, biodiverse forests that are resilient to climate change (Rodrigues et al. 2009). Moreover, the engagement of governmental stakeholders highlights how scientific research, and its potential solutions, extend beyond the academic sphere into the public domain, influencing decision-making processes and contributing to the development of public policies (Kim et al. 2023). Nevertheless, the selection of nucleation techniques by stakeholders often varies according to their specific objectives and, most importantly, the previous land use of the areas in which restoration projects are implemented (Fig. 2B and S4B).

Our findings reveal notable differences in the use of nucleation techniques across land-use types. In abandoned pastures, techniques that integrate both fauna- and flora-related approaches were predominant, highlighting their complementary roles in ecosystem recovery. Such techniques, as e.g. artificial perches and brushwood are particularly effective in these areas, where they enhance connectivity between degraded landscapes and forest fragments (Reis et al. 2010). Artificial perches attract seed-dispersing animals, while brushwood provides shelter, help suppress invasive grasses, and fosters favorable conditions for plant establishment, all of which contribute to natural regeneration processes (Toso et al. 2020; Fragoso et al. 2021). Flora-related techniques, such as seedling islets, further enhance restoration by improving microhabitats for seed germination, offering shade, moisture, and protection (Wilson et al. 2021), thus promoting ecological succession. Over time, these islets can also attract seed-dispersing fauna, especially when native species that provide food resources are planted, reinforcing landscape connectivity and supporting ecological recovery. However, abandoned pastures in the Neotropical region often face significant challenges posed by invasive grasses, which impede natural regeneration and hinder ecological restoration efforts (Elgar et al., 2014; Holmes and Matlack 2019; Rojas-Botero et al., 2020). Addressing these challenges requires targeted restoration strategies that combine multi-trophic level techniques with effective management of invasive species.

In contrast, soil-focused techniques, such as topsoil transfer, are more commonly applied in areas impacted by mining (Amaral et al. 2016; Barbosa et al. 2022; Fonseca et al. 2024). These methods have proven highly effective in accelerating vegetation recovery and

restoring soil microbiota, especially when natural cover is removed prior to ore extraction. Topsoil transfer can improve soil fertility as well as support biodiversity recovery by facilitating the reestablishment of plant and microbial communities essential for long-term ecosystem stability (Darmody et al. 2009; Barbosa et al. 2022; Miguel et al. 2023).

2.6.3 Spatial patterns and quantitative parameters of nucleation

The nuclei size, density, spacing, and the total area occupied by nucleation projects are interconnected variables. We observed that these variables were significantly influenced by stakeholders, land use types, and the focus of the nucleation techniques. These findings highlight how spatial patterns in nucleation projects are heterogeneous, reflecting not only these primary factors but also a combination of other contextual elements, such as available resources, site scale, desired outcomes, and labor requirements (Wilson et al., 2021).

When the private sector is involved, projects often aim to balance ecological objectives with financial costs while meeting legal requirements. In turn, when researchers and governmental agents are involved, the focus is often on testing new methodologies and ecological theories, contributing to knowledge generation and its integration into public policies. This collaboration contributes to ensuring scientific validation as well as to scaling-up the application of successful practices across diverse contexts.

Despite these contributions, the variability in spatial and quantitative patterns highlights the lack of clear guidelines for using nucleation techniques as strategies for ecological restoration. Unlike more established methods, such as sapling planting, nucleation lacks robust standardization. The primary existing guide, the *Applied Nucleation Guide* (Wilson et al., 2021), provides recommendations solely for seedling islands (referred to as applied nucleation) (Corbin and Holl, 2012; Zahawi et al., 2013; Holl et al., 2020). This gap hinders the broader adoption of nucleation in public policies and limits its potential to significantly contribute to large-scale restoration efforts (Holl et al., 2024). For nucleation to become a widely recognized restoration strategy, it is essential for different stakeholders to collaborate in developing accessible and consistent methodologies aligned with practical needs and policy objectives (McGuire and Perna, 2023).

While certain gaps persist, our findings offer valuable insights that could guide the standardization of spatial and quantitative parameters in nucleation practices. For example, soil restoration techniques are often characterized by high-density and closely spaced nuclei,

particularly in mining areas where rapid recovery is a priority. In contrast, fauna-focused techniques typically employ larger, more widely spaced nuclei, especially in contexts such as natural regeneration and forestry. These configurations aim to enhance landscape connectivity and facilitate animal movement. Such variations not only reflect distinct ecological objectives but also underscore the influence of specific constraints and resource availability tied to different land-use contexts. Nuclei spacing showed considerable variability, influenced by both academic research-based practices and private sector strategies that balance costs with ecological goals. Parameters such as vegetation restoration, soil improvement, and habitat creation for fauna require different spatial configurations and are closely tied to land use types, as observed in this study (Figs. 3 and 4).

Finally, the involvement of scientific researchers in nearly all analyzed publications underscores their central role in advancing nucleation as a restoration strategy. This reflects a gradual yet promising transition from small-scale experiments to large-scale restoration projects (Fig. 3D). These increasingly integrated projects involve multiple stakeholders and are applied across diverse land-use contexts, highlighting the potential of nucleation techniques to become widely adopted for the restoration of degraded areas in the Neotropics.

2.7 Conclusions

Our findings show that nucleation techniques are used by various stakeholders and across diverse land-use types in restoration projects. Despite some progress, standard techniques still dominate, with limited innovation or combined approaches. We identified quantitative and spatial patterns in Neotropical nucleation projects that could inform guidelines to standardize practices and support large-scale restoration efforts. Advancing the use of combined techniques remains essential to optimize spatial efficiency and deliver diverse ecological solutions for restoration challenges.

2.8 Acknowledgments

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3. Chapter 2: Using nucleation strategies to promote Atlantic Forest's species and functional diversity during early restoration

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3.1 Abstract

The Atlantic Forest, a global biodiversity hotspot, is highly endangered with only 12% of its original cover remaining. Restoration efforts are growing but remain challenged by these land-use changes. One promising approach is nucleation techniques, which involve creating vegetation nuclei to aid natural colonization and improve conditions for plant establishment. This study examines the impact of nucleation techniques on forest restoration in abandoned tropical pastures during early recovery stages. We hypothesized that increasing the gradient and varying the focus of nucleation (soil, flora, fauna and combined) would improve both taxonomic and functional diversity. Nine nucleation techniques were tested, following a gradient of diversity, with treatments including one technique, three techniques from the same group, three techniques from different groups (one from each group), and nine techniques (maximum diversity). After 15 months, we found that some nucleation techniques significantly increased species diversity and ecological functionality, with techniques involving seed inputs, such as seed nests and topsoil transfer, showing the highest plant recruitment. Combining techniques promoted greater plant species diversity, although functional diversity did not yet follow the same pattern. Depending on the focus of each technique, species composition followed different trajectories in the early stages of restoration but showed a trend toward convergence and greater homogeneity over time. We identified key species capable of overcoming environmental filters in the degraded area, with potential for use in restoration projects in contexts with similar land-use histories. These findings highlight the potential of nucleation as an efficient and scalable strategy for restoring degraded landscapes.

3.2 Introduction

Global ecosystem degradation has intensified dramatically, largely driven by human activities such as agricultural expansion and deforestation, posing serious risks to biodiversity, ecosystem services, and climate stability (Soh et al., 2019). The increasing frequency and intensity of extreme weather events, such as prolonged droughts, severe storms, and heat waves, are closely linked to these human-induced changes and their degradation of natural systems (Pörtner et al., 2023). These changes not only diminish the resilience of ecosystems but also impact human health and well-being. This underscores the urgent need to restore degraded ecosystems, as highlighted by global initiatives like the UN Decade on Ecosystem

Restoration (2021-2030) and the Bonn Challenge, which aims to restore 350 million hectares of degraded land by 2030 (Abhilash, 2021). Such large-scale restoration efforts are essential to building climate resilience, enhancing biodiversity, and safeguarding essential ecosystem services.

One of the priority areas for ecological restoration in Brazil is the Atlantic Forest biome, which has experienced severe degradation since its exploitation began over 500 years ago. The degradation of the Atlantic Forest resulted primarily from agricultural expansion, deforestation, cropping, and real estate speculation. The Atlantic Forest is recognized by the Convention on Biological Diversity (CBD) as one of the 36 global biodiversity hotspots due to its exceptional biodiversity, high levels of endemism, and vulnerability to degradation (Eisenlohr et al., 2015). Currently, only about 12% of its original cover remains, making it the most threatened biome in Brazil and home to 72% of the country's population (SOS Mata Atlântica, 2024). Restoration efforts in the Atlantic Forest are becoming increasingly common, however, a major challenge is posed by large areas of exotic pasture dominated by the *Urochloa* genus (commonly known as brachiaria) and the *Melinis* genus (fat grass). These invasive grasses form dense mats that can inhibit or prevent natural regeneration and impede the establishment of native species in restoration projects (Sartorelli and Campos-Filho, 2017).

In this context, ecological restoration serves as a vital tool that, through human intervention, aims to rapidly reestablish ecosystem processes, including interspecific interactions and ecological succession (Brancalion et al., 2015). One approach to restoration is nucleation techniques, based on nucleation theory (Yarranton and Morrison 1974). These techniques are designed to restore degraded areas by forming vegetation nuclei that facilitate the natural colonization of additional plant species and improve establishment conditions (Reis et al., 2014). The nuclei can function as steppingstones in degraded open areas, facilitating animal movement and promoting seed dispersal. This is a crucial aspect during the restoration of highly degraded areas, as the arrival and deposition of diaspores are key processes in ecosystem recovery. These processes are driven by the interaction between plants and seed-dispersing animals, which play an essential role in forest regeneration and the formation of tropical forests (Carlo and Morales, 2016; Reid and Holl, 2013). Over time, these vegetation islands are expected to expand and merge, ultimately forming a continuous forest.

Incorporating a trait-based approach and functional diversity (FD) into restoration planning is crucial to overcome barriers for ecosystem recovery (Piaia et al., 2023). This is particularly important in abandoned grasslands dominated by invasive exotic grasses, where selecting species with competitive traits, such as rapid growth or dense canopy formation, is essential (Mazzochini et al., 2024). Restoration practices that prioritize FD not only improve the establishment of native species but also facilitate the recovery of ecosystem processes for long-term success (Mori et al., 2013). One way to promote FD in the early stages of restoration is through the use of various nucleation techniques (Rojas-Botero et al., 2020). Additionally, FD provides a more precise understanding of how diversity relates to ecosystem processes, resilience, and services, making it an effective metric for measuring restoration progress (Piaia et al., 2023).

An integrative review of nucleation techniques revealed that the most effective approach to restoration involves combining multiple techniques (Bahia et al., 2023). This is because, depending on the chosen technique and species selection, restoration can follow varying trajectories (Stuble et al., 2017). Balancing these factors is crucial for optimizing the cost-benefit ratio while achieving the desired ecosystem restoration (Bechara et al., 2021; Cruz et al., 2020; Rojas-Botero et al., 2020). Nucleation techniques provide a flexible and effective strategy for ecological restoration, allowing for targeted interventions across multiple trophic levels, such as improving soil conditions, restoring flora, and attracting animals to the area under restoration. These techniques not only initiate ecological processes but also shape the trajectory of recovery, fostering conditions that support natural interactions and ecosystem dynamics (Fraser et al., 2015; Loch et al., 2020; Rojas-Botero et al., 2020). Monitoring these trajectories, including trophic interactions and functional diversity, is essential for evaluating restoration success and ensuring that ecosystems progress toward desired states, rather than merely focusing on vegetation recovery (Fraser et al., 2020). By integrating nucleation techniques with a trajectory-based approach, restoration efforts can better align with ecological principles, enhancing the resilience and long-term sustainability of restored ecosystems.

This study investigates the effects of nucleation strategies on promoting forest restoration in abandoned tropical pastures during the early stages of recovery. We hypothesized that increasing the gradient of nucleation techniques, along with varying the focuses of such techniques, would enhance both taxonomic and functional diversity. To test these hypotheses, we addressed the following research questions: (i) Are the species and

functional diversity of regenerating plants differentially affected by the identity of the nucleation techniques? (ii) Does the focus of the nucleation technique, as well as its isolated versus combined use, influence restoration success? (iii) Do species and functional diversity increase with the gradient of nucleation techniques? (iv) Does the restoration trajectory change depending on the focus of the nucleation technique used? (v) Are there differences between the restoration approaches applied in the study area (i.e. nucleation, traditional sapling planting, and the biodiversity enrichment “*BEF Atlantic*”) in terms of richness, diversity, and composition of the regenerating plant communities?

3.3 Materials and Methods

3.3.1 Study area

The study was conducted in an area of Atlantic Forest at the experimental farm of the *Núcleo de Integração Acadêmica para Sustentabilidade Socioambiental* (NIASSA), SE Brazil (21°34'58.70"S 43°34' 02.5"W). The farm encompasses 240 ha, including 47 ha of native forest, characterized as a remnant of Seasonal Semideciduous Forest and areas of natural regeneration, along with approximately 193 ha of abandoned pastures, predominantly composed of *Urochloa* sp. (Figure 6). The land-use history of the farm includes coffee cultivation until the late 19th century, followed by cattle ranching and the planting of exotic pastures until 2013 (Vittoretto, 2010). Since 2013, there have been no records of agricultural activities on the property. The soil is classified as Dystrophic Red-Yellow Latosol (UFV, 2010). The region's climate is classified as humid subtropical climate with dry winter and hot summer (Cwa), according to the Köppen classification (Alvares et al., 2013).

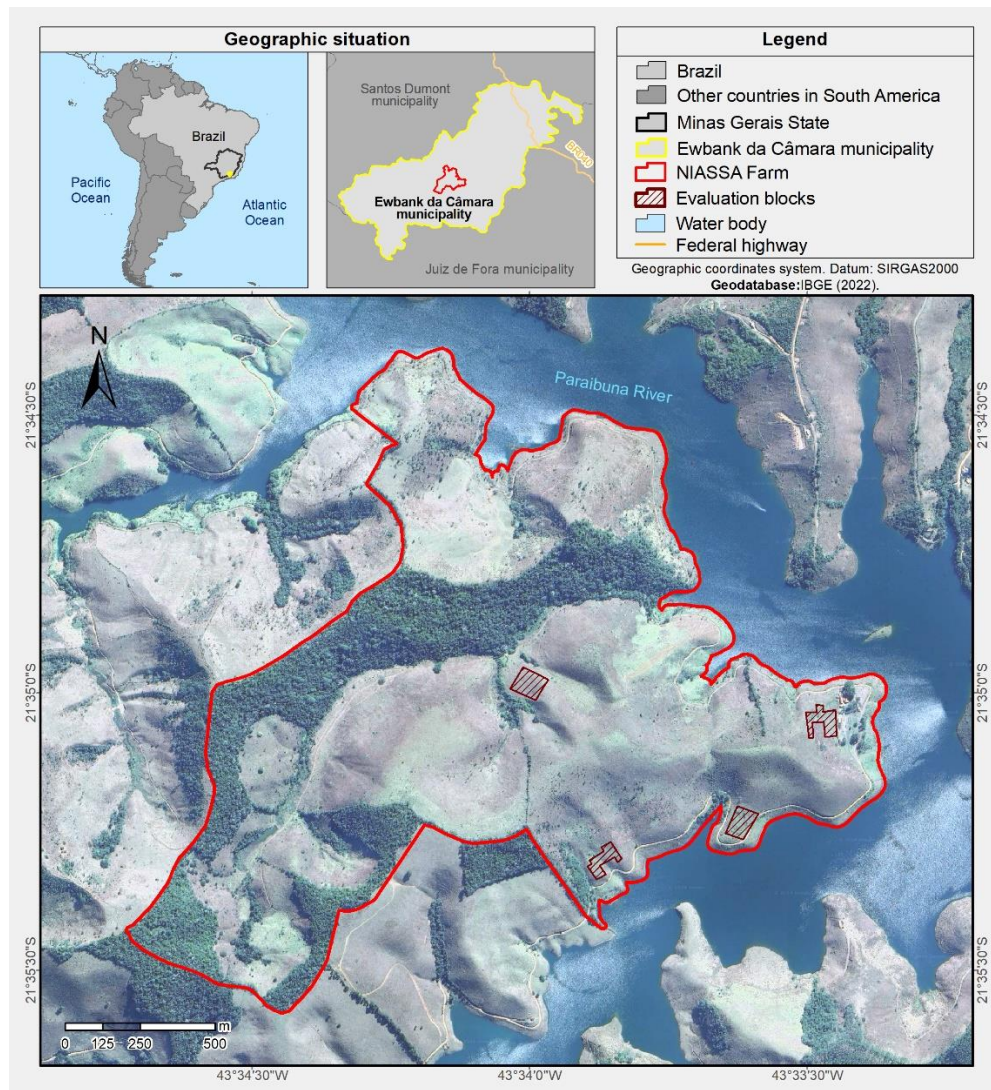


Figure 6. Location of the four experimental blocks (in white) within the NIASSA/UFJF farm (in red).

3.3.2 Nucleation techniques

To test the effectiveness of nucleation strategies in promoting the recovery of species of trees native to the Atlantic Forest in an area of abandoned pasture, we employed nine nucleation techniques, grouped according to their primary objectives into three categories: (1) fauna attraction, (2) soil restoration, and (3) vegetation enhancement. For the fauna attraction group, three techniques were selected: T-perch, hovel perch, and brushwood. For the soil restoration techniques, we selected the translocation of topsoil from mature forest, translocation of topsoil from an area undergoing natural regeneration (hereafter, regeneration topsoil), and litter transfer from mature forest. The techniques aimed at vegetation

enhancement included seedling islets, direct seeding, and seed nests. Below is a detailed description of each technique used.

T-perch: A structure with a height of 4 meters (3.5 meters exposed and 0.5 meters buried) and two landing surfaces, each 1 meter in length, placed 2.7 meters and 3.3 meters above the ground. The purpose of this structure is to provide a landing area for birds, and through defecation and regurgitation, these animals deposit seeds beneath the perches, contributing to the formation of diversity nuclei (Reis et al., 2014).

Hovel perch: A structure approximately 4 meters in height, made of three joined and buried bamboo poles, providing multiple landing areas.

Brushwood: A grouping of branches, pruning residues, and trunks, approximately 1m³ in volume, aimed at providing shelter for animals, including seed-caching species. It also serves as a microclimate area conducive to seed germination, where the plant material decomposes, enriching the soil and supplying nutrients to plants (Reis et al., 2014).

Forest topsoil: This technique involves removing the topsoil layer from a conserved fragment and depositing it in the area to be restored. The aim is to bring forest soil containing edaphofauna, microorganisms, and the seed bank to the restoration site (Reis et al., 2014). Portions of forest soil measuring 0.50 x 0.50 x 0.10 cm were collected and placed in the experimental area.

Regeneration topsoil: This technique is similar to the one described above, but the soil is removed from a fragment of forest in natural regeneration, aiming to bring a seed bank of early-succession species to the restoration site.

Litter transfer: This technique involves collecting only fresh and partially decomposed forest litter from a forest in an advanced successional stage and depositing it in the experimental plots.

Seeding islet: Dense planting of seedlings in a cross shape, with a spacing of 0.50 m between plants. This consists of five plants, with four pioneer species and one non-pioneer species placed in the center. The pioneer species used were *Schinus terebinthifolia* (Brazilian pepper tree), *Cecropia pachystachya* (embaúba), *Citharexylum myrianthum* (pau-viola), and *Handroanthus heptaphyllus* (ipê-roxo), while *Nectandra oppositifolia* (canela) was used as the late secondary species. In total, 320 individuals were planted, with 256 pioneers and 64 late secondary species.

Direct seeding: Direct seeding of native species for coverage and diversity, recommended for the region, was carried out, along with the seeding of pigeon pea (*Cajanus cajan*) due to

its potential as a green manure, nitrogen fixation, rapid growth, and ground cover. Coverage species are characterized by fast growth, good canopy coverage, and attractiveness to fauna, while diversity species are slower-growing and tend to advance in the forest succession process (Brancalion et al., 2015). A total of 14 species were used for direct seeding, with 70 seeds per replication (five seeds of each species). The coverage species included *Enterolobium contortisiliquum* (Vell.) Morong (timbaúva), *S. terebinthifolia*, *Trema micrantha* (L.) Blume (grandiúva), *Mimosa caesalpiniiifolia* Benth. (sansão-do-campo), *Schizolobium parahyba* (Vell.) Blake (guapuruvu), *Peltophorum dubium* (Spreng.) Taub. (canafístula) and *C. myrianthum*. For the diversity group, the species used were *Cariniana estrellensis* (Raddi) Kuntze (jequitibá-branco), *Myrsine coriacea* (Sw.) R.Br. ex Roem. & Schult. (capororoquinha), *Astronium graveolens* Jacq. (guaritá), *Ceiba speciosa* (A.St.-Hil.) Ravenna (paineira), *Cordia superba* Cham. (babosa-branca), *Ormosia arborea* (Vell.) Harms (olho-de-cabra) e *Colubrina glandulosa* Perkins (sobrasil).

Seed nest: We used the same seed mix and direct seeding methodology, and subsequently covered the seeds with forest litter from the forest fragment.

3.3.3 Experimental design

To evaluate the effects of identity, the focus and the diversity of nucleation techniques used (i.e., the gradient of nucleation techniques) on species and functional diversity during the early stages of Atlantic Forest restoration, we established a field experiment in an abandoned pasture that had been left fallow for approximately 10 years. The experiment consisted of 18 treatments (Table 1), utilizing nine nucleation techniques arranged along a gradient of technique diversity. The treatments included combinations of a single nucleation technique, three techniques with the same focus, three techniques with different focuses (one from each focus group), and all nine techniques used simultaneously (i.e., maximum diversity) (Table 1 and Figure 7). For all treatment combinations, the aboveground biomass, primarily composed of braquiária grass, was removed through weeding and herbicide application before the implementation of the nucleation techniques. In the control treatment, the same initial procedure as for the implementation of the nucleation techniques was applied; however, no subsequent management or enrichment actions were carried out during the experiment. In the fallow treatment, the area was simply demarcated, with no interventions performed, thus keeping the cover of *Urochloa* species as initially observed.

Table 1. Nucleation techniques, treatments, and combinations used.

Treatment	Technique			Abbreviation
T1	T-perch			TP
T2	Hovel perch			HP
T3	Brushwood			BR
T4	Forest topsoil			FT
T5	Regeneration topsoil			RT
T6	Litter transfer			LT
T7	Seedling islet			SI
T8	Direct seeding			DS
T9	Seed nest			SN
T10	Control			COM
T11	Fallow			FAL
	3 Techniques - same focus			
T12	TP	HP	BR	FAUNA
T13	SI	DS	SN	FLORA
T14	FT	RT	LT	SOIL
	3 Techniques - different focus			
T15	BR	FT	SN	MIX1
T16	TP	LT	DS	MIX2
T17	HP	RT	SI	MIX3
	Maximum diversity			
T18	TP	HP	BR	ALL
	FT	RT	LT	
	SI	DS	SN	

The sampling effort comprised 72 experimental plots measuring 17 x 17 m, distributed across four experimental blocks of 18 plots each (Figure 6). Within each plot, 9 subplots of 1 m² were established, spaced 3.5 m apart (Figure 7), where nucleation techniques were applied. In total, 648 subplots of 1 m² were used.

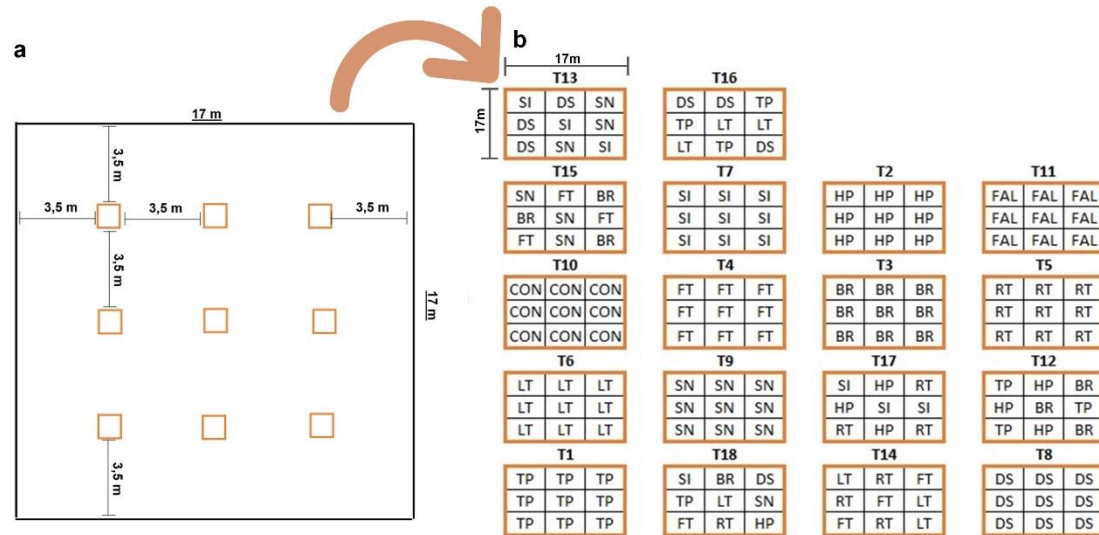


Figure 7. A. Plot design of 17 x 17m with 9 subplots of 1m²; B. Experimental block design with all treatments.

3.3.4 Data collection

Each subplot was monitored at three- or four-month intervals over a 15-month period following the implementation of the nucleation techniques. The study assessed seedling emergence and survival, identifying individuals to the lowest possible taxonomic level, whether species or morphospecies (Zwiener et al., 2014; Cruz et al., 2020; Rojas-Botero et al., 2020). Additionally, all recruited and planted individuals were measured for height and basal diameter (BD). The inclusion criterion for recruitment measurement and identification was set for plants with a height ≥ 50 cm. The evaluation focused on the woody species observed in the experiment.

Seven functional traits were analyzed for the recorded species, being them leaf area in cm² (LA), specific leaf area in cm².g⁻¹ (SLA), thickness in mm, leaf dry-matter content (LDMC, mg.g⁻¹), proportional leaf Nitrogen content, proportional leaf Phosphorus content and dispersal syndrome. These traits were selected as proxies for key ecological functions, including resource-use efficiency, biomass production, stress tolerance, and dispersal mechanisms (Pérez-Harguindeguy et al., 2013). Together, these seven traits were used to calculate functional dispersion (FDis), providing insights into trait diversity and ecosystem

functioning. The data were mostly collected in the field, following the protocol proposed by Pérez-Harguindeguy et al., (2013), but the TRY database and publications were also used (Kattge et al., 2020; Aidar et al., 2003). To construct the data matrix, the mean values of the measured traits were calculated for each species and treatment.

3.3.5 Data analysis

The Shannon-Wiener index, Gini-Simpson index, and Pielou's evenness were calculated to compare the nucleation techniques, nucleation focus, and the techniques used alone or combined, using the *vegan* package (Oksanen et al., 2013). Functional diversity, measured through functional dispersion (FDis), was calculated using the *FD* package (Laliberté et al., 2014). To build the functional space reflecting species' functional trait values, the Gower distance matrix was applied. This method accommodates diverse variable types, such as binary and numeric, ensuring equal weighting for all (Laliberté and Legendre, 2010).

To assess how the identity of different nucleation techniques, their focuses, and their use in combination affected the Gini-Simpson and the FDis diversity indices, we applied generalized linear mixed-effects models (GLMMs) using the *glmmTMB* package (Brooks et al., 2023). Such models included either the identity of the nucleation techniques, their focuses and whether they were applied alone or in combination as a fixed factor, and a random intercept for the experimental blocks. To avoid pseudoreplication, the measurements collected from each 17×17 m plot, which consisted of nine 1 m^2 subplots, were grouped according to the treatment combinations. The control and fallow treatments were not included in these analyses. Values of the Gini-Simpson and the FDis indices were log-transformed ($\log_{10}$) to fulfil the assumptions of the analysis (i.e., normality of residuals and homogeneity of variances). To test whether species and functional diversity of regenerating plants increased with the gradient of nucleation technique diversity, we also applied generalized linear mixed-effects models (GLMMs) using the *glmmTMB* package (Brooks et al., 2023). These models had either the Gini-Simpson or the FDis indices as response variables in function of the gradient of nucleation techniques as a fixed factor and included experimental blocks as a random intercept. While the model assessing the Gini-Simpson index also included the control plots as part of the gradient of nucleation technique diversity (from 0 to 9; transformed by $\log_{10}[x + 1]$) to allow for testing the effects of natural regeneration (i.e., zero nucleation techniques applied), the model testing FDis excluded control and fallow plots (gradient of nucleation technique diversity from 1 to 9; transformed by $\log_{10}$) because no trait values could

be obtained from such plots, as no species were recruited in these treatments. Again, data from all subplots were pooled to avoid pseudoreplication, and the response variables were log-transformed to fulfill the assumptions of the analysis. For all models, the significance of the fixed factors was determined through a Wald Test (type 2) calculated with the function *Anova* of the *car* package (version 3) in R (Fox and Weisberg, 2019). Statistical analyses were performed in R version 4.3.1 (R Core Team 2023). In all cases, significant effects were assessed using pairwise comparisons conducted with the *emmeans* package (Lenth et al., 2018). Model performance was assessed graphically and with the R-package *DHARMA* (Hartig, 2020).

To assess the restoration trajectory through community composition over time according to the focus of the nucleation techniques, we used non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity. To evaluate differences in abundance and richness across the three restoration strategies implemented on the NIASSA farm (i.e., nucleation, traditional sapling planting, and biodiversity enrichment 'BEF Atlantic'), we used a Generalized Linear Model (GLM) with a Gaussian family, implemented through the *lme4* package. Significant effects were assessed using pairwise comparisons with the *emmeans* package (Lenth et al., 2018). Richness and abundance data for recruits were standardized based on the duration of each project. Recruitment rates (richness and abundance) were calculated by dividing the observed values (recruits) by the time elapsed since the experiment or planting began: 1.5 years for nucleation, 3 years for traditional sapling planting, and 3 years for BEF. The continuous nature of these rates (non-integer values) justified the use of a Gaussian family in the GLM for statistical analyses.

3.4 Results

In total, 274 arborescent and arboreal individuals were recruited, representing 17 species from eight families. Fabaceae was the most representative family with six species, followed by Euphorbiaceae and Solanaceae with three species each. The most abundant species was *Solanum mauritianum* Scop. (N = 121), followed by *Schizolobium parahyba* (Vell.) Blake (N = 43), *Aegiphila integrifolia* (Jacq.) Moldenke (N = 28) and *Trema micranthum* (L.) Blume (N = 24). These four species represent 75% of the total abundance recorded in all nucleation treatments. Among the nucleation treatments, the most effective were the seed nest (SN), which recruited 66 individuals across six species, the forest topsoil (FT), with 51 individuals from eight species, and the soil combination (SC), which resulted in

47 individuals across seven species (Figure 8). The control (C), fallow (FA) and hover perch (HP) treatments did not present any recruited plants so far. T-perch (TP) presented only one individual, represented by *Miconia albicans* (Sw.) Steud.

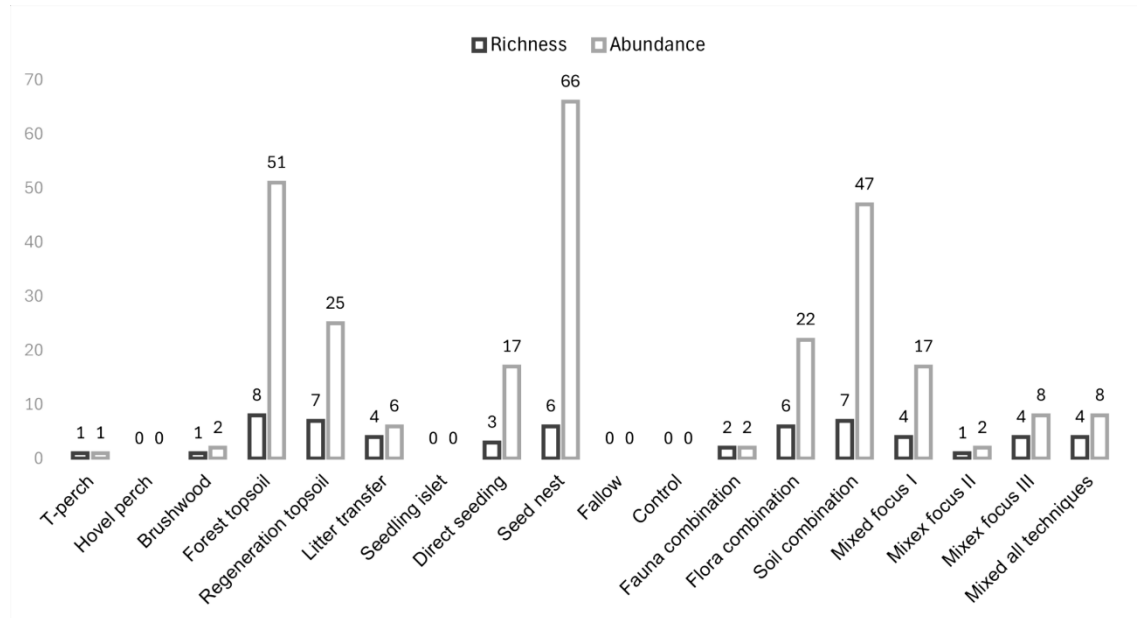


Figure 8. Richness and abundance of plants recruited in different nucleation techniques.

A total of 323 individuals were recruited over a 15-month period, with recruitment rates ranging from 27% to 177% across field campaigns (Table 2). When extrapolated to one hectare per year, this corresponds to a 4,456.49 individuals·ha⁻¹·year⁻¹. Mortality rates varied between 10% and 15%, with an overall mortality rate of approximately 10% for the entire experiment (Table 2).

Table 2. Recruitment rate, mortality, and number of plants recruited in the intervals between campaigns over 15 months of monitoring.

Intervals (months)	Recruit rate	Mortality rate	Nº of recruit (ha ⁻¹ ·year ⁻¹)
C1-C2 (4)	71.11%	9.89%	3,101.05
C2-C3 (4)	81.25%	14.07%	2,407.41
C3-C4 (3)	176.92%	14.69%	6,111.11
C4-C5 (3)	27.17%	11.90%	1,543.21

Regarding diversity indices, species recruitment was significantly influenced by the treatment, as indicated by the Shannon index, Gini-Simpson index (Figure 9A) and Pielou index (Table 3). When considering the focus of the technique (soil, flora, fauna, or multiple), a significant effect was observed on the Shannon index and Pielou index, while no significant difference was found for dominance (Table 3 and Figure 9B). Similarly, comparisons between techniques applied individually or in combination did not show a significant effect on the diversity indices tested in relation to plant recruitment (Table 3 and Figure 9C).

Table 3. Diversity indices associated with species recruitment in nucleation treatments, considering three analytical approaches: (i) different nucleation treatments, (ii) different technique focuses, and (iii) comparison between individually applied and combined techniques. The diversity indices analyzed were Shannon, Gini-Simpson, and Pielou. The values presented correspond to the chi-square test (χ^2), degrees of freedom (df), and p-values (p). Statistically significant differences ($p < 0.01$) are marked with '*' on p-values.

Nucleation treatment			
Diversity index	X²	df	p
Shannon	144.52	15	<0.01*
Gini-Simpson	44.52	15	<0.01*
Pielou	140.50	15	<0.01*
Focus technique			
Shannon	10.02	2	<0.01*
Gini-Simpson	0.92	2	0.62
Pielou	9.85	2	<0.01*
Techniques individually vs combination			
Shannon	0.56	1	0.45
Gini-Simpson	0.98	1	0.32
Pielou	0.64	1	0.42

In terms of functional diversity, the functional dispersion index (FDis) showed a significant difference between treatments ($X^2 = 53.55$; $df = 13$; $p < 0.01$) (Figure 9D). When considering the focus of the technique and the techniques applied individually or in combination, these did not show a significant effect on FDis (Figure 9E and F).

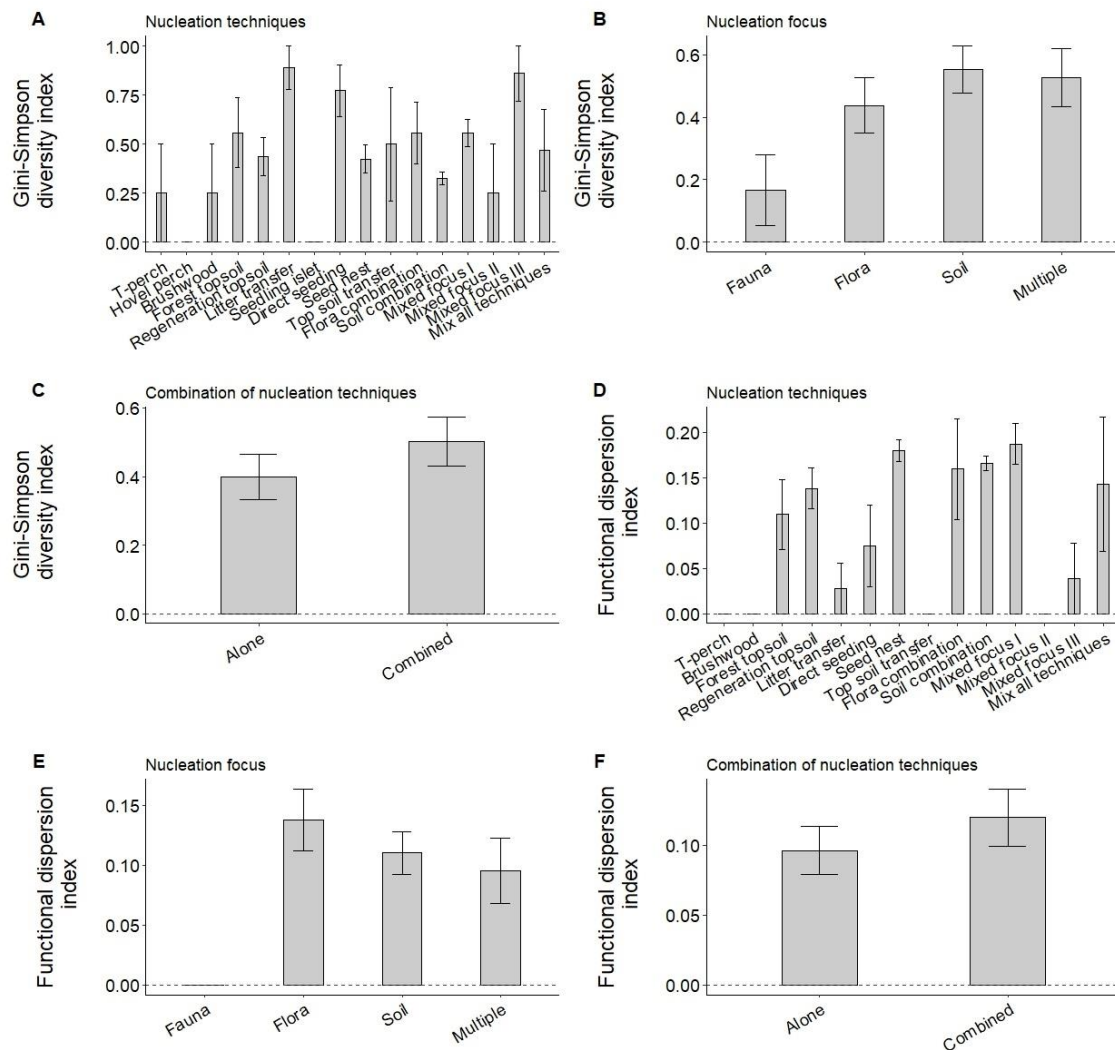


Figure. 9. Gini-Simpson diversity index and functional dispersion index for the nucleation techniques used, the effect of combining techniques versus using them alone, and the focus of the nucleation technique applied. (A) Nucleation techniques: Gini-Simpson diversity index for the different nucleation techniques. (B) Nucleation focus: Gini-Simpson diversity index categorized by the focus of the nucleation technique (fauna, flora, soil, or multiple). (C) Combination of nucleation techniques: Gini-Simpson diversity index comparing treatments with techniques used alone versus combined. (D) Nucleation techniques: Functional dispersion index for the different nucleation techniques. (E) Nucleation focus: Functional dispersion index categorized by the focus of the nucleation technique (fauna, flora, soil, or multiple). (F) Combination of nucleation techniques: Functional dispersion index comparing treatments with techniques used alone versus combined.

The gradient of nucleation techniques showed a difference in the Gini-Simpson index ($X^2 = 4.15$; $df = 1$; $p < 0.05$), indicating a trend where a higher gradient of techniques is associated with greater diversity (Figure 10A). However, for functional diversity, the FDis did not differ with respect to the gradient of techniques used (Figure 10B).

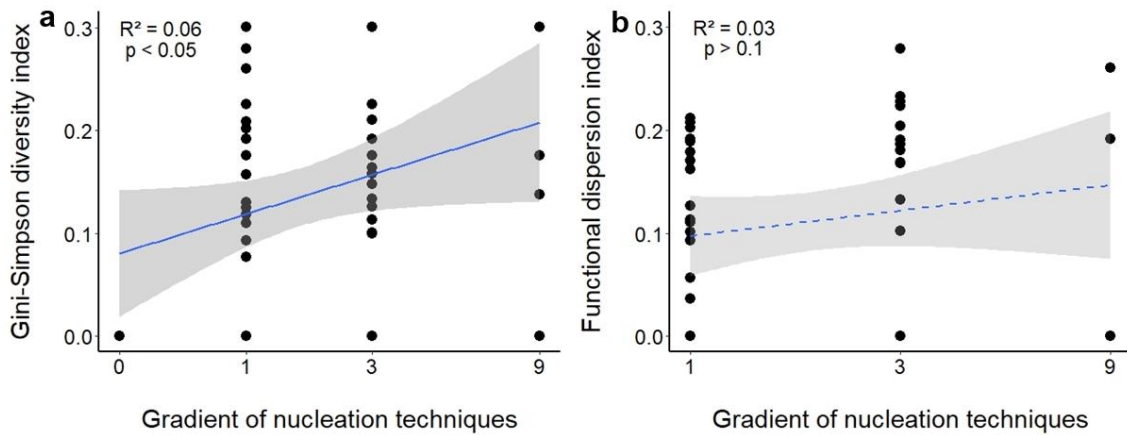


Figure 10. Relationship between the gradient of nucleation techniques and the (A) Gini-Simpson diversity index and (B) functional dispersion index. The nucleation gradient includes control areas with 0 techniques, treatments with one nucleation technique, treatments with three techniques, and treatments with 9 techniques.

The non-metric multidimensional scaling (NMDS) ordination revealed distinct trajectories for the nucleation techniques over time, based on Bray-Curtis dissimilarity (Figure 11). The ordination plot showed that techniques focusing on soil restoration and those integrating multiple approaches exhibited close clustering, suggesting minimal shifts in community composition. Nucleation techniques focusing on flora showed distinct trajectories over time in the NMDS ordination. Early time points (March and August 2023) were more separated along the NMDS1 axis, indicating differences in community composition compared to other nucleation focus groups. Over time, more recent points (January and April 2024) became closer to the soil and multiple nucleation groups. In contrast, the fauna remained static across all time points, with all observations overlapping in the NMDS space, as only one species (*Miconia albicans*) was recorded, which is also present in the flora group (Figure 11).

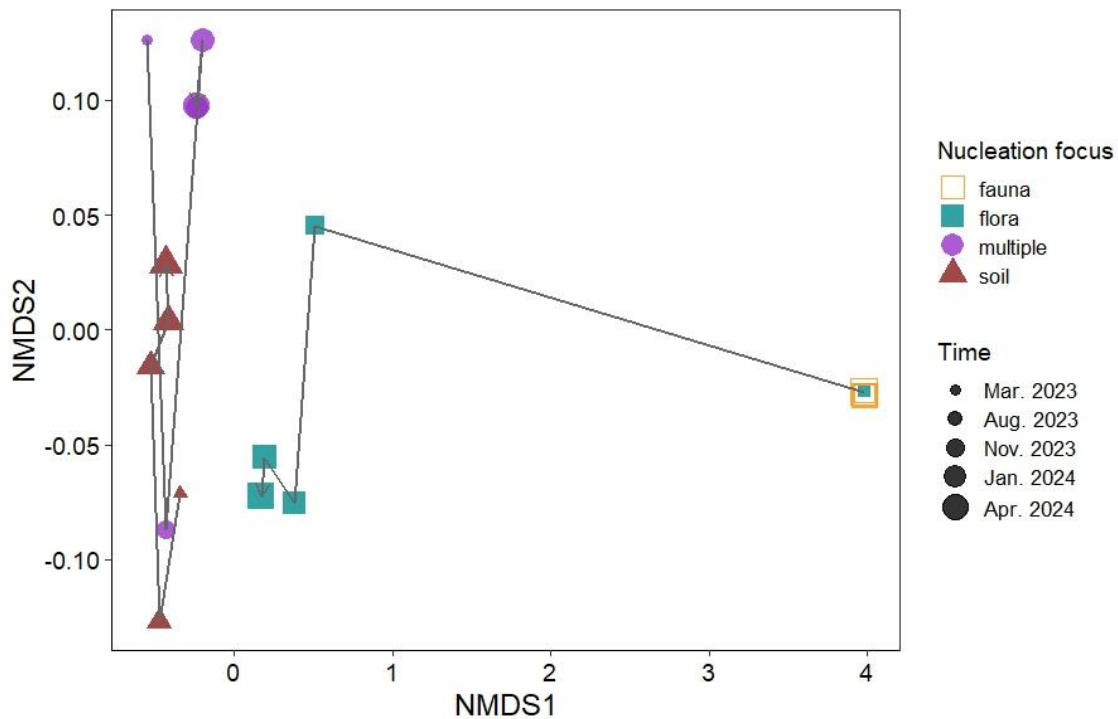


Figure 11. Changes in species composition according to the nucleation focus (soil, fauna, flora, or multiple) in abandoned tropical pasture over 15 months, based on NMDS (Stress: 0.1%). The trajectories are represented by symbols according to the sampling time. Different nucleation focuses are indicated by different shapes and colors: fauna (square, orange), flora (square, blue), multiple (circle, purple), and soil (triangle, red).

The analysis of species abundance and richness in the three restoration strategies, nucleation, traditional tree planting and biodiversity enrichment (BEF Atlantic), indicated significant differences between the strategies for both variables ($p < 0.03$). Tukey's post hoc test revealed that nucleation was different from BEF ($p < 0.01$) and traditional ($p < 0.02$) for richness and also for abundance ($p < 0.01$; $p < 0.02$, respectively) (Figure 12). It is important to highlight that the restoration strategies differ in age. Traditional planting and BEF are approximately 36 months old, whereas nucleation is only 15 months old.

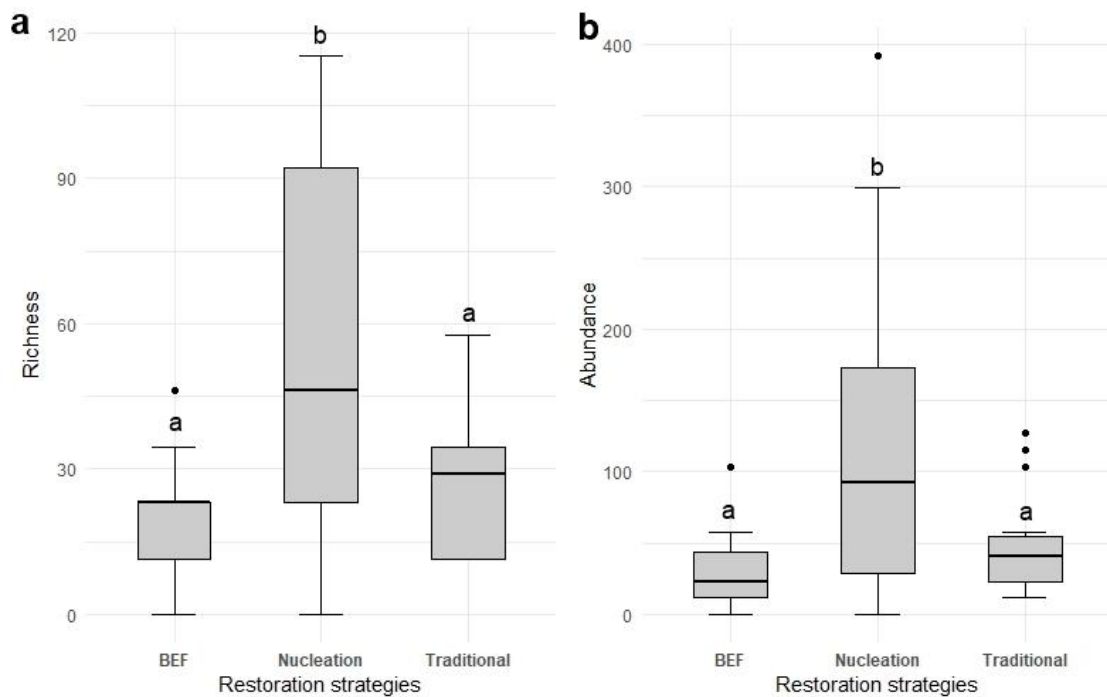


Figure 12. (A) Richness e (B) Abundance across different reforestation methods. The nucleation strategy shows significantly higher values compared to the others for both richness and abundance. Different letters above the bars indicate statistically significant differences between the methods (BEF = biodiversity and ecosystem functionality, traditional planting, and nucleation).

3.5 Discussion

We found that both nucleation treatments and the focus of the techniques influenced species diversity and functional diversity. When considering the isolated versus combined application of nucleation techniques, no significant differences were observed in either diversity index. Regarding the gradient of nucleation techniques, as the complexity of nucleation increases with the addition of different approaches, a trend emerges suggesting that a greater gradient of techniques is associated with higher species diversity, although no similar effect was detected for functional diversity. Additionally, the restoration trajectory differed depending on the focus of the technique applied, with soil-focused and multi-focused treatments following one trajectory, while fauna- and flora-focused treatments formed another trajectory.

Among the evaluated techniques and their combinations, certain approaches stood out, particularly those focused on soil improvement and vegetation enhancement, such as seed nest, forest topsoil, regeneration topsoil, and combinations involving soil and flora. These techniques associated with these focus groups achieved higher species diversity, as indicated by indices such as Shannon, Gini-Simpson, and Pielou. This suggests that the specific mechanisms linked to these techniques, such as improvements in soil structure and the introduction of organic material like leaf litter, likely played a role in influencing seedling recruitment. The importance of topsoil from preserved ecosystems in restoration projects is well-established and widely recognized (Darmody et al., 2009; Barbosa et al., 2022; Bahia et al., 2023; Figueiredo et al., 2024). This surface soil is enriched with a diverse microbiota, a robust seed bank, and favorable physicochemical conditions, which facilitate the establishment of plant species (Parreira et al., 2022). Forest leaf litter is also an efficient source of organic matter and can contribute to microorganisms, soil fertility, physical protection, and propagules (Posada et al., 2012; Caldeira et al., 2019). Additionally, the combined use of topsoil transposition, direct seeding, and litter has been shown to be highly effective in restoration efforts (Figueiredo et al., 2024). Notably, all techniques and combinations that demonstrated the best results incorporated at least one of these components, emphasizing the importance of topsoil, litter, and direct seeding in promoting ecological recovery.

The functional dispersion index (FDis) followed a similar pattern to that observed for species diversity. However, FDis was notably higher in combined techniques involving flora and soil, as well as in multi-focus approaches (e.g., mixed focus I and mix all techniques). This pattern suggests that integrating techniques targeting different trophic levels, such as flora, fauna, and soil, promotes greater functional diversity, potentially driven by niche complementarity provided by the combination of these strategies. Different nucleation techniques create a heterogeneous environment that supports the establishment of a wide array of species with diverse functional traits (Rojas-Botero, 2020; Piaia et al., 2023). Plant growth within nucleation patches tends to create a more heterogeneous environment due to the varied species compositions, seedling ages, and environmental conditions (Corbin and Holl, 2012). This heterogeneity reduces interspecific competition and fosters greater niche partitioning, aligning with the ecological theory of niche complementarity (Tilman, 1999). Niche complementarity also enhances the relative abundance of species, which in turn increases functional diversity. According to Mason et al. (2013), the functional divergence

index (FDiv) tends to rise as niche complementarity boosts the relative abundance of species. Although no statistically significant differences were detected among the focus techniques or their combinations in this study, the high FDis values observed highlight the potential of approaches integrating multiple trophic levels and distinct techniques. Such strategies generate environmental heterogeneity and enhance ecosystem functionality (Figure 9).

In the present study, the Gini-Simpson diversity index showed a positive relationship with the gradient of nucleation techniques, demonstrating an increase in species diversity as the number of combined techniques increased (Figure 10A). This finding suggests that more diversified nucleation strategies create heterogeneous environments that facilitate the recruitment of a broader range of species while ensuring more balanced abundance distributions. However, the functional dispersion index (FDis) did not follow the same pattern, as no significant relationship was observed between FDis and the gradient of nucleation techniques (Figure 10B). While species diversity can recover relatively quickly following a disturbance, the restoration of functional diversity tends to be a slower process. This is because functional diversity depends not only on species recruitment but also on the establishment of species with distinct ecological functions that can occupy different niches within the ecosystem (Cianciaruso et al., 2009).

Aligned with plant species composition, the trajectory analysis revealed distinct communities at the early stages of restoration. However, over time, certain groups, particularly those associated with multiple focus approaches and soil restoration techniques, exhibited increasing similarities in plant community structure (Figure 11). Additionally, soil-focused techniques exhibited the least displacement within the NMDS space, indicating that the composition of recruited species remained more stable over time. In contrast, techniques emphasizing flora enhancement displayed distinct community compositions and were associated with fauna recruitment through only a single species, *Miconia albicans*.

Despite the current differences in plant communities recruited across nucleation focus techniques, the observed trajectories suggest a trend toward convergence. This pattern likely reflects the influence of environmental filters in this degraded ecosystem, dominated by the invasive alien species *Urochloa sp.* The rapid growth of this invasive grass poses significant challenges to restoration efforts (Fragoso et al., 2021). For example, *Urochloa sp.* was reported to reinvade restoration areas as early as eight months after the implementation of nucleation techniques, disrupting native species establishment and compromising restoration success (Fragoso et al., 2020).

The different focus techniques in nucleation likely contributed in distinct ways to species recruitment, potentially influenced by factors such as soil fertility, water availability, and the specific mechanisms associated with each technique. Environmental filters in the degraded area, including competition for nutrients and space with the dominant *Urochloa sp.*, soil compaction, intense sunlight exposure, limited nutrient availability, and herbivory pressure, further narrow the pool of species capable of establishing in such challenging conditions (Hobbs and Norton 2004). As a result, these filters often lead to a convergence in community composition over time (Rojas-Botero et al, 2020). This is evidenced by the recruitment of only 17 species under nucleation techniques, from a regional pool of approximately 400 woody species (Borges et al., 2021). Even so, nucleation techniques and their combinations promoted the recruitment of these key species, which are extremely important to be used in restoration in landscapes with the same land use history and characteristics.

A similar pattern of early differences in community composition followed by subsequent convergence was also observed by Rojas-Botero et al. (2020) in degraded areas dominated by invasive alien species, where different nucleation techniques were applied. The authors suggest that environmental filters play a dominant role in shaping restoration trajectories during the early stages, reinforcing the importance of tailored strategies to overcome these constraints.

Among the three restoration strategies evaluated in this study, using richness and abundance of recruited plants as success metrics, nucleation was the most efficient compared to traditional seedling planting and biodiversity enrichment (BEF). It is important to highlight that the richness and abundance data for nucleation corresponds to a period of 15 months, while the values for BEF and traditional planting correspond to 36 months. When adjusted for this time difference, the results for nucleation become even more impressive, demonstrating a superior relative efficiency in a shorter period.

The high recruitment observed in nucleation treatments is likely related to the specific techniques used, particularly those that involve seed inputs, such as direct seeding, seed nests, and topsoil transfers. These methods directly introduce viable seeds or propagules into the environment while also enhancing conditions for seedling establishment through the addition of organic matter and beneficial microorganisms from the topsoil (Navarro-Pedreño et al., 2017; Figueiredo et al., 2021). Traditional restoration strategies, such as BEF and traditional planting, tend to focus solely on seedling planting and may present slower initial recruitment

when compared to nucleation (Bechara et al., 2021). Furthermore, in tropical forest areas restoration strategies have shown a greater influence on plant recruitment than surrounding forest cover (Holl et al., 2017), highlighting the fundamental role of well-designed interventions in accelerating recruitment processes and promoting the early establishment of species in restoration trajectories. Nonetheless, forest recovery remains a long-term effort, spanning several decades and progressing at highly variable rates, even with active restoration efforts.

3.6 Conclusion

This study demonstrated that nucleation increases both species diversity and ecological functionality in abandoned pastures. The most effective nucleation techniques were those associated with the introduction of organic material or seeds, such as topsoil addition, soil combination, and seed nest. This effect was influenced by the identity and focus of the techniques employed, showing that as the diversity of techniques increases, there is a proportional rise in species diversity, although not yet in functional diversity. Depending on the focus of each technique, species composition followed distinct trajectories during the early stages of restoration but showed a tendency toward convergence and greater homogeneity over time. We identified key species capable of overcoming the environmental filters in this degraded area, which can be strategically used in other restoration projects in regions with similar histories of land use and degradation. Nucleation strategies, especially those aimed at seed input and arrival, demonstrated higher plant recruitment compared to other restoration approaches, proving their efficiency even during the early stages of environmental recovery. These findings underscore the potential of nucleation as a robust and scalable strategy for accelerating the restoration of degraded landscapes.

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3.8 Supplemental file: Chapter 1

Supporting information to Freitas, Teixeira et al., Assessing nucleation strategies and spatial dynamics in the restoration of Neotropical terrestrial ecosystems: A comprehensive review

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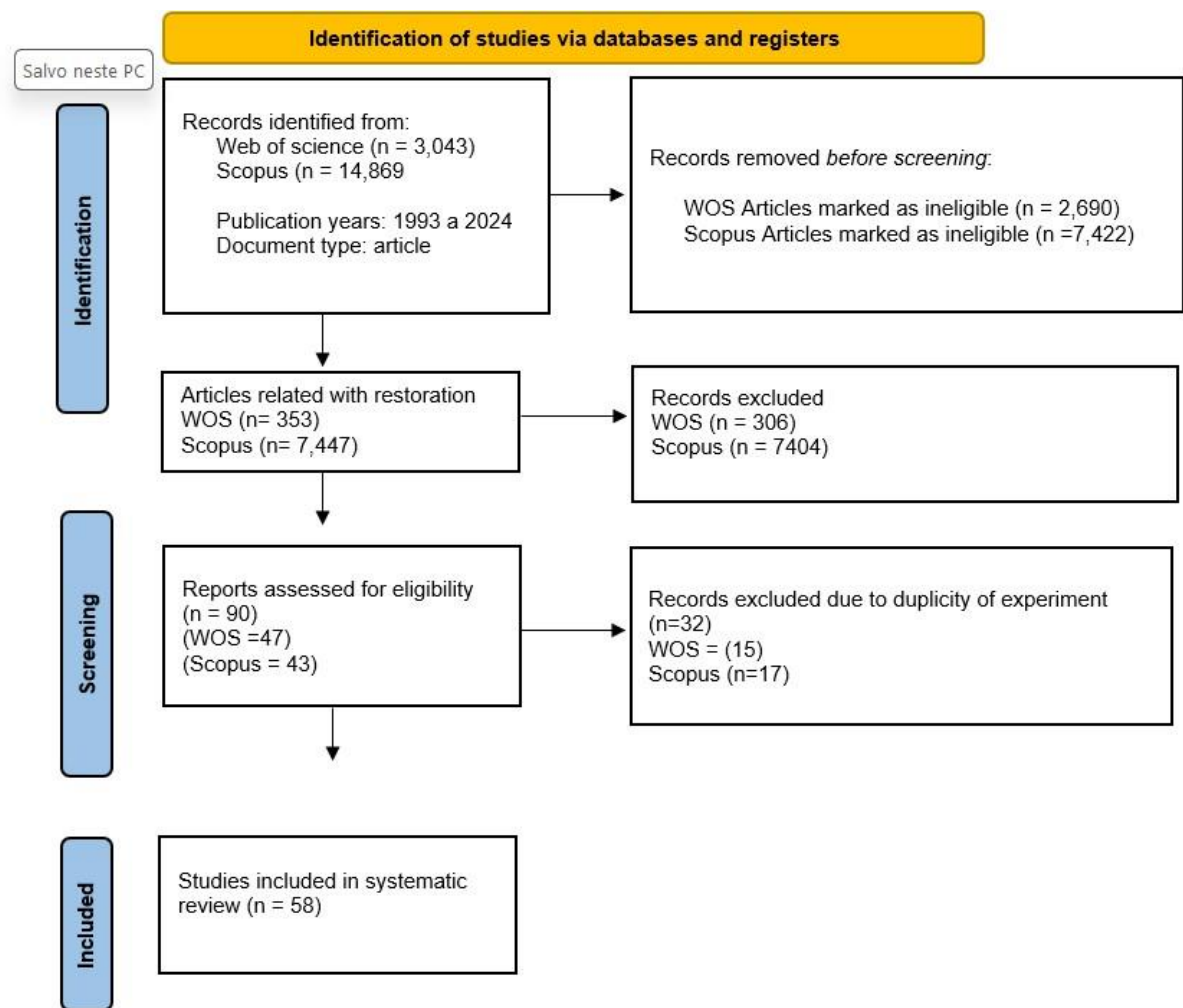


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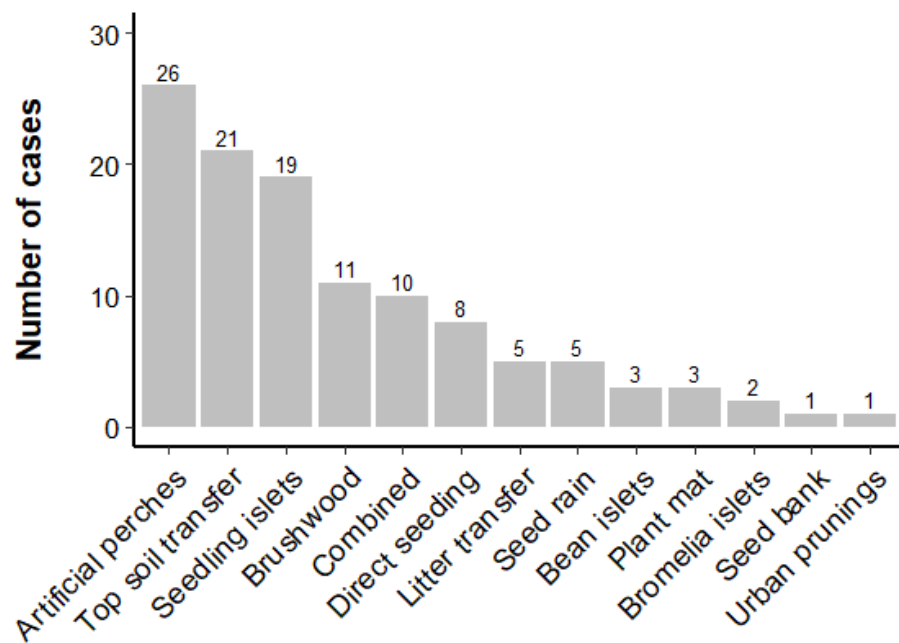


Figure S2: Frequency of nucleation techniques used in the studies included in the systematic review.

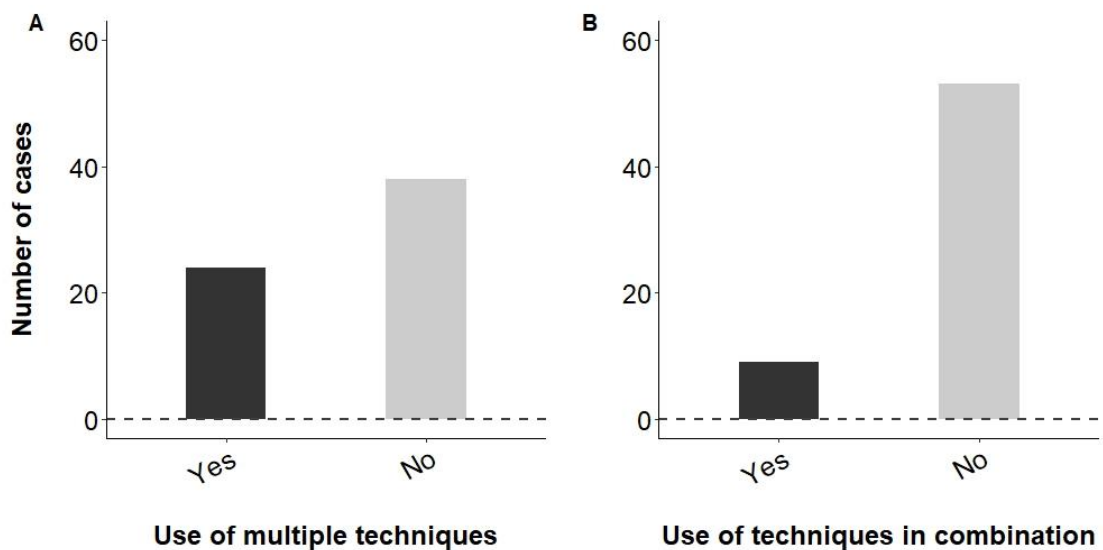


Figure S3: Frequency of multiple-to-single and combined-to-alone nucleation studies included in the systematic review.

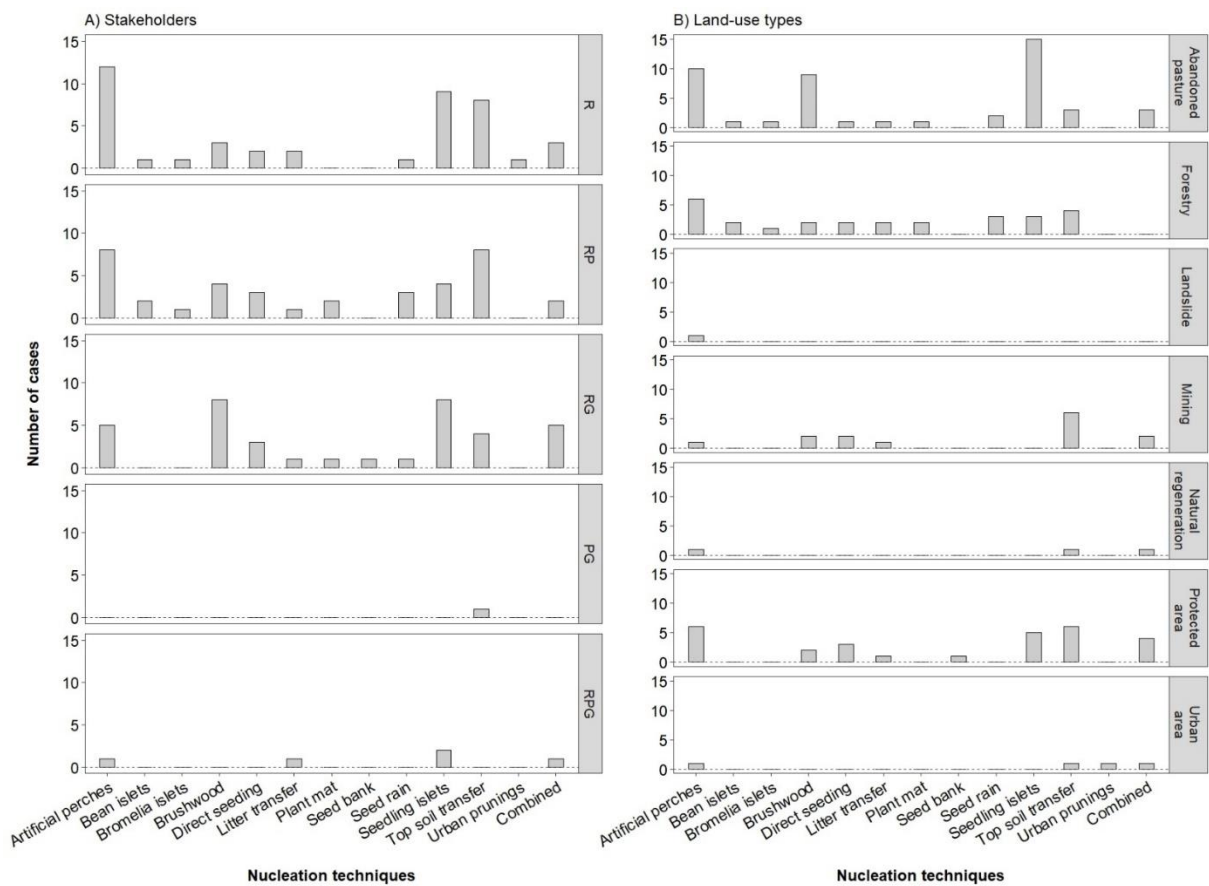


Figure S4: Frequency of nucleation techniques used in studies according to stakeholders (A) and Land-use types (B).

Table S1: List of studies included in the systematic review after selection.

Id number	Author (s)	Year	Title	Source	Country
1	Almeida A, Marques MCM, Ceccon-Valentec MF, Vicente-Silva J, Mikich SB	2016	Limited effectiveness of artificial bird perches for the establishment of seedlings and the restoration of Brazil's Atlantic Forest	Journal for Nature Conservation	Brazil
2	Amaral LA, Pereira IM, Silva MAP, Oliveira MLR, Machado ELM, Laia ML	2017	Use of topsoil for restoration of a degraded pasture area	Pesquisa Agropecuária Brasileira	Brazil
3	Amaral LA, Pereira IM, Silva MAP, Machado ELM, Gonzaga APD, Kneg RAP	2016	Viability of using topsoil ferruginous yoke in the restoration of a waste dump	Bosque	Brazil

4	Araujo IC, Dziedzic M, Maranhão LT	2014	Management of the environmental restoration of degraded areas	Brazilian Archives of Biology and Technology	Brazil
5	Athiê S, Dias MM	2016	Use of perches and seed dispersal by birds in an abandoned pasture in the Porto Ferreira state park, southeastern Brazil.	Brazilian Journal of Biology	Brazil
6	Barbosa, RD; do Vale, RS; Schwartz, G; Martins, WBR; Ribeiro, SS; Rodrigues, JID; Ferreira, GC; Barbosa, VM	2022	Restoration of degraded areas after bauxite mining in the eastern Amazon: Which method to apply?	Ecological Engineering	Brazil
7	Barrera-Cataño, JI; Garibello, J; Moreno-Cárdenas, C; Basto, S	2023	Trade-offs at applying tree nucleation to restore degraded high Andean forests in Colombia	Restoration Ecology	Colombia
8	Bechara, FC	2006	Unidades demonstrativas de restauração ecológica através de técnicas nucleadoras: Floresta Estacional Semidecidual, Cerrado e Restinga.	Dissertation	Brazil
9	Bechara, FC; Trentin, BE; Engel, VL; Estevan, DA; Ticktin, T	2021	Performance and cost of applied nucleation versus high-diversity plantations for tropical forest restoration	Forest Ecology and Management	Brazil
10	Bertoncello R.; Oliveira A.A.; Holl K.D.; Pansonato M.P.; Martini A.M.Z.	2016	Cluster planting facilitates survival but not growth in early development of restored tropical forest	Basic and Applied Ecology	Brazil
11	Brandão JFC, Martins SV, Brandão IJ, Lopes WP	2017	Ecological restoration in area dominated by <i>Pteridium aquilinum</i> (L.) Kuhn in Caparaó National Park, MG	Revista Árvore	Brazil
12	Castelli KR , Barreto MG, Francesconi W, Valle LD , Mondelli G, Abilio FM , Silva A M	2015	Analysis of effectiveness of three forest interventionist techniques and proposal of a new and integrated model of forest restoration	Environmental Technology	Brazil
13	César RG, Viani RA, Silva Candido da M, Brancalion PH	2014	Does a native grass (<i>Imperata brasiliensis</i> Trin.) limit tropical forest restoration like an alien grass (<i>Melinis minutiflora</i> P. Beauv.)?	Tropical Conservation Science	Brazil
14	Cruz DC, Benayas JMR, Ferreira GC, Ribeiro SS	2020	Tree Communities in Three-Year-Old Post-Mining Sites Under Dierent Forest Restoration	Forests	Brazil

Techniques in the Brazilian Amazon					
15	de Freitas TC, Gomes GC, Molina AR, Guarino ESGG, Agra CI, Beltrame R.	2022	Artificial perches increase bird-mediated seed rain in agricultural fallow area in southern Brazil	Web Ecology	Brazil
16	Dias CR, Umetsu F, Breier TB	2014	Contribution of artificial perches to seed dispersal and its application to forest restoration	Ciência Florestal	Brazil
17	Ferreira GA, Melo C	2016	Artificial roosts as seed dispersal nuclei in a Cerrado area in triângulo mineiro, Brazil	Bioscience Journal	Brazil
18	Figueiredo M.A.; Messias M.C.T.B.; Leite M.G.P.; Kozovits A.R.	2021	Direct seeding in the restoration of post-mined campo rupestre: Germination and establishment of 14 native species	Flora: Morphology, Distribution, Functional Ecology of Plants	Brazil
19	Fonseca, WD; Martins, SV; Fiorese, EM; Villa, PM	2024	Complementing seedling planting with nucleation techniques increases forest restoration potential in areas around bauxite mining	Land Degradation & Development	Brazil
20	Fragoso, RD; Carpanezzi, AA; Zuffellato-Ribas, KC; Koehler, HS	2017	Forestry restoration in abandoned pastures of <i>Urochloa</i> by different sizes of brushwood	Cerne	Brazil
21	Fragoso RO, Carpanezzi AA, Zuffellato-Ribas KC, Koehler HS	2020	Brushwood and artificial perch did not facilitate forest regeneration in <i>Urochloa</i> spp. Grassland	Brazilian Journal of Botany	Brazil
22	Fragoso RD, Carpanezzi AA, Zuffellato-Ribas KC, Koehler HS	2021	Forest restoration by different nucleation techniques in <i>Urochloa</i> grassland	Pesquisa Agropecuária Brasileira	Brazil
23	Holl KD	1998	Do Bird Perching Structures Elevate Seed Rain and Seedling Establishment in Abandoned Tropical Pasture	Restoration Ecology	Costa Rica
24	Holl KD, Reid JL, Cole RJ, Oviedo-Brenes F, Rosales JA, Zahawi RA	2020	Applied nucleation facilitates tropical forest recovery: Lessons learned from a 15-year study	Journal of Applied Ecology	Costa Rica
25	Iguatemy MD, Vilarinhos JA, Oda GAM, Conde MDS, Zaú AS	2020	Artificial Perches: Ecological and Functional Aspects of its Contribution in the Atlantic Forest	Floresta e Ambiente	Brazil
26	Leal-Filho N, Santos GR, Ferreira RL	2013	Comparing nucleation techniques used for the restoration of degraded areas in the Brazilian Amazon	Revista Árvore	Brazil

27	Loureiro, N; de Souza, TP; do Nascimento, DF; Nascimento, MT	2022	Survival, seedlings growth and natural regeneration in areas under ecological restoration in a sandy coastal plain (restinga) of southeastern Brazil	Austral Ecology	Brazil
28	Marcuzzo SB, Ganade G, Araújo MM, Muniz MFB	2013	Comparison of nucleation techniques effectiveness for degraded area restoring in southern Brazil.	Floresta	Brazil
29	Martins D.A.P.; Lanzarini A.C.; Heinz C.F.; Vieira F.S.; Bonatto R.A.; Kanieski M.R.	2017	Evaluation of the litter and soil seed bank transposition in a degraded area in the “Planalto Catarinense” region.	Floresta	Brazil
30	Melo VA, Griffith JJ, de Marco Junior P, Silva E, Lopes de Souza A, Carneiro Guedes M, Faria Ozório T	2000	Efeito de poleiros artificiais na dispersão de sementes por aves	Revista Árvore	Brazil
31	Neto, AM; Kunz, SH; Martins, SV; Silva, KD; da Silva, DA	2010	Transposition of soil seed bank as a methodology of forest restoration of abandoned pasture in Vicos, MG	Revista Árvore	Brazil
32	Oliveira AJF	2013	Recuperação de uma área degradada do cerrado através de modelos de nucleação, galharias e transposição de banco de sementes	Thesis	Brazil
33	Onésimo C.M.G.; Dias D.D.; Vale Beirão M.; Kozovits A.R.; Messias M.C.T.B.	2021	Ecological succession in areas degraded by bauxite mining indicates successful use of topsoil	Restoration Ecology	Brazil
34	Piaia, BB; Rovedder, APM; Procknow, D; Camargo, B; Gazzola, MD; Croda, JP; Stefanello, MD	2020	Natural regeneration as an indicator of ecological restoration by applied nucleation and passive restoration	Ecological Engineering	Brazil
35	Piaia, BB; Rovedder, APM; Procknow, D; Fockink, GD; Camargo, B	2023	Taxonomic and functional diversity of applied nucleation for ecological restoration	Plant Ecology	Brazil

36	Piaia BB, Rovedder AP, Costa EA, Felker RM, Piazza EM, Stefanello MD	2017	Transposition of the seed bank for ecological restoration of seasonal forest in Rio Grande do Sul, Brazil	Revista Brasileira de Ciências Agrárias	Brazil
37	Piaia B.B.; Rovedder A.P.M.; Procknow D.; Camargo B.	2021	Assessment of ecological indicators in restoration by applied nucleation with different ages	Ciência Florestal	Brazil
38	Pillatt N, Pillatt N, Franco ET, Coelho GC	2010	Dry artificial perches and the seed rain in a subtropical riparian forest	Revista Brasileira de Biociências	Brazil
39	Pilon NAL, Buisson E, Durigan G	2018	Restoring Brazilian savanna ground layer vegetation by topsoil and hay transfer	Restoration Ecology	Brazil
40	Pilon NAL, Assis GB, Souza FM, Durigan G	2019	Native remnants can be sources of plants and topsoil to restore dry and wet cerrado grasslands	Restoration Ecology	Brazil
41	Procknow D, Rovedder AP, Piaia BB, Camargo B, de Moraes Stefanello M, da Silva MP, da Silva PS, Croda JP, Dreyer JB	2023	Monitoring ecological restoration of riparian forest: Is the applied nucleation effective ten years after implementation in the Pampa?	Forest Ecology and Management	Brazil
42	Rojas-Botero S.; Solorza-Bejarano J.; Kollmann J.; Teixeira L.H.	2020	Nucleation increases understory species and functional diversity in early tropical forest restoration	Ecological Engineering	Colombia
43	Rudge, AC	2008	Contribuição da chuva de sementes na recuperação de áreas e do uso de poleiros como técnica catalisadora da sucessão natural	Dissertation	Brazil
44	Sampaio A.B.; Holl K.D.; Scariot A.	2007	Does restoration enhance regeneration of seasonal deciduous forests in pastures in central Brazil?	Restoration Ecology	Brazil
45	Schorn LA, Krieger A, Nadolny MC	2010	Avaliação De Técnicas Para Indução Da Regeneração Natural Em Área De Preservação Permanente Sob Uso Anterior Do Solo Com Pinus elliottii	Floresta	Brazil
46	Shiels AB, Walker LR	2003	Bird Perches Increase Forest Seeds on Puerto Rican Landslides	Restoration Ecology	Porto Rico
47	Silva MP, Rovedder AP, Hummel RB, Toso LD, Felker RM, Peccatti A, Matiello J	2019	Initial development and phenology in restoration nuclei in the Mata Atlântica biome, Southern Brazil	Revista Brasileira de Ciências Agrárias	Brazil

48	Silveira LP, Souto JS , Damasceno MM, Mucida DP, Pereira IM	2015	Poleiros artificiais e enleiramento de galhada na restauração de área degradada no semiárido da Paraíba, Brasil	Nativa	Brazil
49	Simioni, GF; Schmitt, AL; Joner, F; Farley, J; Fantini, AC; Moreira, APT	2022	Response of birds to high biodiversity silvopastoral systems: Integrating food production and biodiversity conservation through applied nucleation in southern Brazil	Agriculture Ecosystems & Environment	Brazil
50	Tomazi AL, Castellani TT	2016	Artificial perches and solarization for forest restoration: assessment of their value	Tropical Conservation Science	Brazil
51	Tomazi AL, Zimmermann CE , Laps RR	2010	Poleiros artificiais como modelo de nucleação para restauração de ambientes ciliares caracterização da chuva de sementes e regeneração natural	Revista Biotemas	Brazil
52	Toro L, Torres-Romero F, Salinas SM, Avella- Munoz A, Galatowish S, Secchi S, Powers JS	2024	Cost-effectiveness of management strategies in a nucleation experiment in a tropical dry forest	Restoration Ecology	Colombia
53	Toso LD, Rovedder AP, Hummel RB, Peccatti A, Araújo EF, Felker RM, Silva JC, Neuenschwander F	2020	Fauna associated with brushwood transposition in a mining area in the South of Brazil	Floresta e Ambiente	Brazil
54	Tres DR, Reis A	2009	Técnicas nucleadoras na restauração de floresta ribeirinha em área de Floresta Ombrófila Mista, Sul do Brasil	Biotemas	Brazil
55	Valenzuela P.; Arellano E.C.; Burger J.A.; Becerra P.	2016	Using facilitation microsites as a restoration tool for conversion of degraded grasslands to Nothofagus forests in Southern Patagonia	Ecological Engineering	Chile
56	Zahawi RA, Augspurger CK	2006	Tropical Forest restoration: tree islands as recruitment foci in degraded lands of Honduras	Ecological Applications	Honduras
57	Zanini L.; Ganade G.	2005	Restoration of Araucaria Forest: The role of perches, pioneer vegetation, and soil fertility	Restoration Ecology	Brazil

58	Zwiener, VP; Cardoso, FCG; Padial, AA; Marques, MCM	2014	Disentangling the effects of facilitation on restoration of the Atlantic Forest	Basic and Applied Ecology	Brazil
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