The effects of nucleation and species functional traits on tree seedling performance in the early stage of ecological restoration

Adriana P. Manhães
Federal University of Rio de Janeiro

Nino T. Amazonas
São Paulo Botanical Garden

Marcelo P. Pansonato
São Paulo University

Jerônimo B. B. Sansevero
Federal Rural University of Rio de Janeiro

Adriana Martini

`amzmartini@usp.br`

São Paulo University

Dulce Mantuano
Federal University of Rio de Janeiro

Research Article

Additional Declarations: No competing interests reported.

Abstract

The initiation of a new plant community in a restoration area hinge on the performance of seedlings post-planting. This study aimed to evaluate the impact of species functional traits—specific leaf area (SLA), wood density (WD), seed dry mass (SDM), and potential height (H)—on the seedling performance after 18 months of planting, comparing two planting designs (rows and clusters) and the influence of fertilization addition in clusters. The restoration area is a riparian tropical forest located at Caraguatatuba municipality, São Paulo, Brazil. We monitored 3,017 tree seedlings and estimated their survivorship and relative growth rate (RGR) using the diameter, height, and canopy area of the surviving seedlings and the stem biomass for the cluster RGR estimation. Using linear mixed models, we analyzed how the planting designs and the functional traits affect species survival and their RGR. We underscored the significance of slow-growth traits (low SLA, and high SDM and WD) in enhancing species survival, irrespective of the planting design. Whereas, maximizing species growth entails prioritizing seedlings with greater potential height. Cluster survival and growth improved with a greater abundance of species with low values of SDM (i.e., fast-growth species) and low functional divergence (high similarity). Fertilized clusters improved the RGR of slow-growth species. Including functional traits in restoration is advantageous for enhancing seedling performance at the species level, which is an important consideration for restoration practitioners. To optimize applied nucleation, clusters should target functional diversity at this community level to prevent the dominance of competitive species.

1 Introduction

The performance of seedlings after plantation is crucial in establishing a new plant community in restoration sites, as they have high mortality rates in the initial phase of ecological restoration (Grossnickle 2012). As a result of degradation, restoration areas typically present harsh environmental conditions, including intense light, poor soil fertility, and limited water availability. These conditions challenge the growth and survival of newly planted seedlings. Therefore, environmental factors play a significant role in shaping the success of seedlings, with some species struggling to thrive under such stressful circumstances (Charles et al. 2018). Biotic interactions between seedlings also influence their performance after planting. They can shift from positive (facilitation) to negative (competition) relationships, depending on the density of neighboring species and their life history (Maestre et al. 2009). The facilitation relationship is evident when the individual growth rate is enhanced in the presence of a specific neighbor, whereas competition decreases the individual growth (Callaway et al. 2002; Trinder et al. 2013). Nevertheless, biotic interactions between seedlings after planting may only occur when the crown and root of neighboring seedlings overlap. Such interactions can begin earlier if seedlings are planted closer or in a high-density spatial arrangement.

Planting seedlings aggregately in clusters, also known as tree islands or nucleation, is a cost-effective technique used in ecological restoration and has been shown to alleviate stressful conditions for seedlings during their initial stages of development (Bechara et al. 2021; Holl et al. 2020). The nucleation process occurs when new colonizers are established in patches and spread outward through clonal reproduction or

facilitation (Yarranton and Morrison 1974). The effects of abiotic and biotic limitations on seedling establishment may be lower in clusters than in surrounding areas, as clustering ameliorates stressful microclimatic conditions, promotes the colonization of new individuals, and can attract more animal-dispersed species (Corbin and Holl 2012). Facilitation is expected in clusters, as studies have shown that the nucleation strategy can improve or maintain seedling survivorship in the post-plantation stage compared to traditional row plantations (Bechara et al. 2021; Bertoncello et al. 2016; Silliman et al. 2015). Despite row plantation has been linked to a higher growth rate than the applied nucleation strategy, both exhibited similar growth when species identity was included as an explaining factor (Bertoncello et al. 2016; Holl et al. 2011).

Species performance is based on the trade-off between mortality and growth rates and is subject to variations based on species-specific strategies and the availability of natural resources. Fast-growing species display high growth rates but may not necessarily be good survivors, in contrast to slow-growing species that exhibit lower mortality rates, even in stressful conditions (Laughlin et al. 2020). Nutrient-rich soil may favor the growth of highly competitive fast-growing species, while slow-growing species can have lower mortality rates, even in poor soil conditions (Oliveira et al. 2021). In this regard, plant functional traits can explain the capacity of species to acquire and use resources and their ability to compete and regenerate, placing plant species across a continuum of ecological strategies (Poorter et al. 2014; Westoby et al. 2002; Wright et al. 2004). Fast-growing plant species typically exhibit the following soft (i.e., easily measuring) traits: a short leaf lifespan (high specific leaf area [SLA]), softwood (low wood density [WD]), greater height (high potential height [H]), and small seeds (low seed dry mass [SDM]) (Garnier et al. 2016; Poorter et al. 2014).

Studies on the role of functional traits in elucidating post-planting seedlings' individual performance within restoration areas have revealed varying relationships. Reproductive traits (e.g., SDM) were found to be more important than leaf traits in influencing seedling survivorship and growth after 42 months of planting (Martínez-Garza et al. 2013). Physiological leaf traits (e.g., photosynthetic rate and stomatal conductance) associated with nutrient use efficiency better predicted seedling growth than traits associated with water use efficiency and morphological leaf traits (Guimarães et al. 2018). Seedling survival and growth were promoted in species with higher water use efficiency, but correlation strengths varied between the wet and dry seasons (Werden et al. 2018). In a drier environment, wood density was the best predictor of increased seedling survivorship (Werden et al. 2018). Seedlings of shade-tolerant species (i.e., high leaf mass area [LMA] values) grew less when planted in pastures with higher irradiance levels (Toledo-Aceves et al. 2022). Moreover, leaf functional traits weaker explained seedling performance than their initial height in a subtropical rainforest restoration area (Gardiner et al. 2019).

Seedling neighborhood may also influence planted seedling performance when accounting for biotic interactions in aggregated plantations. Analyzing the influence of neighboring ("crowding") on the survival of focal species, Lasky and colleagues (2014) found an increase in survivorship when neighbors were functionally dissimilar (using leaf traits of SLA and leaf dry matter content [LDMC]). This result can be explained by the principle of limiting functional similarity, which states that two individuals sharing the same ecological niche cannot coexist (MacArthur and Levins 1967). In this regard, competition should

modulate the coexistence of neighboring species, leading to a functionally dissimilar community (MacArthur and Levins 1967). However, the specific functional trait of wood density explained the focal species' survivorship independently of the crowding level (Lasky et al. 2014). In such cases, the species that exhibit superior competitive abilities tend to outcompete and displace others, resulting in a trait-similar community (Mayfield and Levine 2010) contrasting to the functionally dissimilar community led by niche differentiation.

Our study aimed to elucidate the role of functional traits on seedling performance by comparing the relationship between different planting designs (row plantations vs. aggregated clusters) and fertilization types in clusters (fertilized vs. non-fertilized) at the onset of tropical forest restoration (0–2 years). In pursuit of these goals, we addressed two main questions. Initially, we sought to determine whether the interaction between planting design (rows or clusters) and species' functional traits might affect seedling performance (growth and survival). We hypothesized that fast-growing species (e.g., high SLA and H but low SDM and WD values) would display higher growth rates when planted in rows than in clusters due to the presence of neighboring individuals at initial stage. In comparison, slow-growing species would present higher survival in clusters, as these species may be favored (facilitation) in such aggregated planting systems. Second, we examined how the functional similarity of clusters (trait mean and range) affects average seedling performance in distinctive communities regarding soil nutrient availability (fertilized and non-fertilized clusters). We expected that improving soil fertility would foster the growth of acquisitive species (i.e., fast-growing), resulting in a cluster community with higher biomass stock. However, slow-growing species were expected to perform better in poorer soil conditions (non-fertilized clusters), improving seedling survival and the functional dissimilarity of the community.

2 Materials and Methods

2.1 Study site

The restoration site encompasses an experimental area spanning 6.5 hectares, featuring a 1.3-kilometer-long and 50-meter-wide riparian zone that has undergone reforestation (Fig. 1). This area is situated alongside the Camburu River and is situated within the Petrobras company, specifically within the Monteiro Lobato Gas Treatment Unit (UTGCA). This site is located on the Southeastern coast of Brazil on the coastal plain between a mountain range (Serra do Mar) and the ocean in the municipality of Caraguatatuba, São Paulo state. The original vegetation of the site is a wet tropical forest established on white sandy soil, locally called *Restinga* forest, part of the Brazilian Atlantic Forest. The area has a humid subtropical climate, with no dry season, and a hot summer (Cfa) with an annual average temperature of 20.6° C. Accumulated annual precipitation is 1,957 mm on average, July being the driest month, having 67 mm precipitation, and January the rainiest month with 268 mm (Alvares et al. 2013). Originally, the soil had a sandy texture but after a landslide occurred in 1967, the original sandy soil was mixed with clayey soil from the slopes of Serra do Mar throughout the entire plain, albeit with varying intensities (Vieira et al. 2015). Before restoration, the area was a pasture for at least the past 50 years, covered by invasive exotic grasses, with some castor (*Ricinus communis*) and guava (*Psidium guajava*) trees.

2.1.1 Experimental design

The tree planting was conducted in 2012 using an experimental design that considered two main factors: planting design (row plantations, and aggregated clusters) and fertilization (chemical fertilizer, organic fertilizer, and non-fertilized seedlings). The two planting systems had different spacing between seedlings, resulting in two distinctive densities of individuals in the immediate neighborhood for each species. Seedlings in traditional row design were planted with a spacing of 2 x 2 m, resulting in a low neighbor density. In the cluster systems, 13 seedlings were planted aggregately, spacing 0.3 m between them; however, these clusters were 5 m apart from each other. The spatial-aggregated cluster design aimed to understand how species could perform under intense biotic interactions, simulating initial conditions in natural regeneration areas. Although there was a high density in the immediate neighborhood of each seedling inside the clusters, there was more space among clusters in a plot (5 m). The average density of seedlings in the row design was approximately 2,088 ind. ha^{-1} (± 32.3 ind.) and in the cluster design was 3,625 ind. ha^{-1} (± 60.9 ind.). Therefore, we emphasized that the two plant designs of row plantations and aggregated clusters (hereafter called "rows" and "clusters") are characterized by low and high immediate neighborhood density, respectively.

We had two distinctive floristic compositions in clusters (clusters A and B) but maintaining the ratio of five pioneers and eight non-pioneer species (Table 1). For chemical fertilization, a 100-gram dose of an NPK ratio of 06:30:06 was applied at the seedling hole, following a recommendation from the previous soil analysis. To maintain this fertilization treatment for the clusters, an NPK fertilizer with a ratio of 20:0:20 was applied to each seedling every three months for two years. Organic fertilizer consisted of the addition of 2 L of humus at the seedling hole. These led to six combinations of factorial treatments (2 planting designs x 3 levels of fertilization) implemented at 20 m × 50 m plots that were grouped into eight blocks (Fig. 1). For more details about the experimental design, see Bertoncello et al. (2016).

Table 1

The number of seedlings monitored from each species in the two types of non-fertilized planting design: row plantations vs. aggregated clusters; and two types of fertilization in clusters (non-fertilized vs. chemically fertilized). P = pioneer and NP = non-pioneer.

Botanic Family	Species name[1]	Cluster Composition	Ecological group	Rows	Clusters	
				non-fertilized	non-fertilized	fertilized
Anacardiaceae	*Schinus terebinthifolia* Raddi	A,B	P	48	82	85
Arecaceae	*Euterpe edulis* Mart.	A,B	NP	49	82	85
Arecaceae	*Syagrus romanzoffiana* (Cham.) Glassman	A	NP	39	40	44
Bignoniaceae	*Jacaranda puberula* Cham.	B	NP	42	42	41
Calophyllaceae	*Calophyllum brasiliense* Cambess.	B	NP	45	42	41
Fabaceae	*Erythrina speciosa* Andrews	A,B	P	48	82	85
Lamiaceae	*Aegiphila verticillata* Vell.	A	P	46	40	44
Lauraceae	*Ocotea pulchella* (Nees & Mart.) Mez	A	NP	44	40	44
Malvaceae	*Eriotheca pentaphylla* (Vell. & K.Schum.) A.Robyns	A,B	NP	51	82	85
Myristicaceae	*Virola bicuhyba* (Schott ex Spreng.) Warb	A	NP	35	40	44
Myrtaceae	*Eugenia astringens* Cambess.	A,B	NP	51	82	85

[1] The R package flora (Carvalho 2020) was used to check the scientific names of plant species according to Flora e Funga do Brasil (2020).

Botanic Family	Species name[1]	Cluster Composition	Ecological group	Rows	Clusters	
				non-fertilized	non-fertilized	fertilized
Myrtaceae	*Eugenia brasiliensis* Lam.	B	NP	40	42	41
Myrtaceae	*Myrcia ilheosensis* Kiaersk.	B	NP	37	42	41
Myrtaceae	*Myrcia multiflora* (Lam.) DC.	A	NP	42	40	44
Pentaphylacaceae	*Ternstroemia brasiliensis* Cambess.	B	NP	47	42	41
Primulaceae	*Myrsine coriacea* (Sw.) R.Br. ex Roem. & Schult.	A,B	P	52	82	85
Sapotaceae	*Pouteria* Aubl.	A	NP	38	40	44
Solanaceae	*Solanum cinnamomeum* Sendtn.	B	P	44	42	41
Verbenaceae	*Citharexylum myrianthum* Cham.	A,B	P	48	82	85
TOTAL				846	1,066	1,105

[1] The R package flora (Carvalho 2020) was used to check the scientific names of plant species according to Flora e Funga do Brasil (2020).

*(*Table 1*)*

To answer our first question, as we were only interested in evaluating the role of planting design on seedling performance, we selected only the non-fertilized plots for both rows and clusters, resulting in 16 total plots (Fig. 1). For our second question, aiming to understand the role of fertilization at a cluster community level, we used the data set only from cluster plots with and without chemical fertilization (Fig. 1). We did not use the plots with the addition of organic fertilizer in our study as it was not maintained during the period of the survey, only applied at the seedling hole during planting.

To mitigate mortality resulting from post-planting stress, all deceased individuals were replanted one month later. Subsequently, 30 days post-replanting, we conducted individual measurements to assess the seedling survival and growth. The range of initial heights of surveyed seedlings spanned from 2 cm to 140

cm. The monitoring survey occurred at two different moments, one month after the replanting (August 2012) and 19 months after it (March 2014). The survey counted a total of 3,017 individuals monitored from 19 species (Table 1). For plant design comparison at the species level, we sampled 1,912 seedlings of which 846 seedlings were from the row design treatment and 1,115 seedlings from non-fertilized clusters. For the analysis at the cluster community level (fertilized and non-fertilized), we used 2,171 seedlings (Table 1).

2.2 Traits collection

We chose to select four functional traits mostly related to the resources-use continuum and the mortality-growth trade-off: (i) specific leaf area (SLA); (ii) wood density (WD); (iii) potential height, and (iv) seed dry mass (SDM). SLA reflects the balance between resource acquisition and conservation, where species with lower SLA values, termed conservative, have leaves with longer lifespans and lower photosynthesis rates (Perez-Harguindeguy et al. 2016; Wright et al. 2004). Species with higher WD negatively explain tree growth but positively influence survivorship (Chave et al. 2009; Kunstler et al. 2016). Plant species with greater potential heights – H (i.e., maximum height at maturity) tend to exhibit enhanced competitive abilities for accessing light and achieving robust growth (Westoby et al. 2002). Finally, species with larger seed dry mass (SDM) often experience improved seedling survival rates, which can contribute to their overall success and persistence in a community (Garnier et al. 2016).

The survey of functional traits was done in September 2022. For this, five individuals per species were randomly selected within the plots. For each tree individual, we collected three leaf samples and one sample of developed wood (stems between 2.5 and 5.0 cm in diameter and around 10 cm in length). The functional traits collection and measuring followed the protocol described in Perez-Harguindeguy et al. (2016). We measured the wet and dry weights of leaves and estimated their leaf area using the software ImageJ (Rasband 2012). We drought the wet leaf samples in a dry chamber at 40 ºC for 3–5 days until constant weight. From these measurements, we estimated the specific leaf area (SLA, $mm^2 . mg^{-1}$) that is the leaf area divided by its dry weight. Using the water-displacement method (Perez-Harguindeguy et al. 2016), we divided the stem wet volume by its dry weight and estimated the wood density (WD, $g. cm^{-3}$). Then, for SLA and WD, we averaged the leaf and stem individual samples per species to build the species traits matrix. For the estimation of seed dry mass (SDM, mg) we used local data from 10 species from dataset of Zimback et al. (2023) and complemented the other species using two main databases: (1) Neotropical Tree Communities Database – TreeCo (available at http://labtrop.ib.usp.br/) and (2) Plant Trait Database – TRY, available at https://www.try-db.org (Fraser 2020; Kattge et al. 2020). For the estimation of potential height (H, m), we used the database from the NeoTropTree – NTT, available at http://www.neotroptree.info/ (Oliveira-Filho 2017) and filtering data from herbarium dataset, available at http://rb.jbrj.gov.br/v2/consulta.php. As the SDM and H traits have less intraspecific variation we could complement our species traits matrix with these secondary datasets.

2.3 Data analysis

2.3.1 Performance

Seedling performance was estimated by measuring survival and growth rate at individual (n = 1,912), species (n = 19), and cluster (n = 167) levels. To calculate the percentage of species survival, we used the binary values of survival (1) and mortality (0) of each individual, but also averaged it per species. For cluster survival, we estimated the percentage of survived individuals for each cluster (13 individuals planted). All survival variables were calculated at 19 months after planting.

We measured the following seedling variables in the survived individuals to estimate growth: (i) height (H, cm); (ii) diameter at ground level (DGL, mm), and (iii) canopy area (CA, cm^2, using an ellipse area from the perpendicular diameters of the canopy). For growth estimation, we used the relative growth rate (RGR) formula from Pommerening and Muszta (2016), as shown in the equation below:

$$RGR = \frac{\left(log\frac{Y_k}{Y_{k-1}}\right)}{\Delta t}$$

in which, Y_k is the seedling variable at the final time and Y_{k-1} at the initial time, Δt is the period of analysis (in our case Δt = 18 months), leading to an RGR per month unit.

Then, the RGR at the individual level was averaged by species. For the RGR at the cluster level, we used its total aboveground biomass – AGB (stem in Kg) using the equation described in Chave et al. (2005), as following:

$$AGB = F\text{x}\rho\text{x}\left(\frac{\pi D^2}{4}\right)\text{x}H$$

in which, F is the tree taper that we used F = 0.033 considering a uniform shape (conical shape); ρ is de wood density of species (g.cm^{-3}); we used DGL (converted to cm) for tree diameter (D); and H is the individual height (converted to m) (Chave et al. 2005).

2.3.2 Functional traits

Using the species traits matrix, we estimated the value of the community-weighted mean (CWM) at the cluster level. CWM is the average of the functional trait weighted by the relative dominance of each species (using basal area). After 19 months of planting, we calculated three independent functional diversity indices: functional richness – FRic, functional divergence – FDiv, and functional evenness – FEve (Mouchet et al. 2010). They cover three groups of functional diversity as described by Garnier et al. (2016), which are: richness (related to taxonomic richness and does not take species abundance into account), divergence (functional distance between pairs of species considering their abundance), and equity (demonstrates inequalities in distribution attributes along the functional axis, also weighted by species abundance). For this, we used the *FD* package in R (Laliberté et al. 2014).

2.4 Statistical analysis

To assess differences in seedling survival and relative growth rate (RGR)—based on seedling height, diameter, and canopy area—between row and cluster planting designs, we used generalized linear mixed-effect models. The models incorporated the planting design as a fixed effect and block as a random intercept. For analyzing the impact of species functional traits on their survival and growth, the models included treatment, species trait values, and their interaction as fixed effects, with blocks as random intercepts. Due to potential multicollinearity, as some traits showed high correlations (see Online Resource 1), a single model was executed for each functional trait (SLA, SDM, WD, and H).

To evaluate the effect of chemical fertilization on seedling survival and growth (biomass RGR) at the cluster-community level, another set of generalized linear mixed-effect models was applied. These models considered the treatment of fertilization, the community trait values (community-weighted mean – CWM, and functional diversity – FD indices), and their interactions as fixed effects, with blocks as random intercepts. Single models were run for each community trait value to avoid issues arising from inter-index correlations (Online Resource 1).

Models with survival as the response variable were fitted with a binomial distribution, while those for RGR (at species and cluster levels) assumed a normal distribution. All leaf traits (SLA, LDMC) and seed dry mass required log transformation to satisfy normality assumptions. These analyses were conducted using linear mixed-effects models in the R Core Team (2021) employing the lme4 package (Bates et al. 2009).

3 Results

3.1.1 Seedling performance

We found that seedling performance (individual level) was significantly different between the planting designs (rows and clusters without fertilization) and fertilization types (non-fertilized vs. fertilized clusters). After 19 months of planting, seedling survival in rows ($45.5 \pm 49.8\%$) was lower ($z = 3.876$; $P < 0.001$) than the planting in clusters ($53.6 \pm 49.9\%$). However, the RGR for diameter (DGL) was higher ($t = -8.94$; $P < 0.001$) in rows (0.065 ± 0.036 mm per month) than clusters (0.046 ± 0.037 mm per month). Moreover, despite any significant effect of chemical fertilization in seedling survival planted in clusters ($z = -1.781$; $P = 0.075$), RGR was improved for all variables (DGL: $t = 6.823$; $P < 0.001$, H: $t = 2.626$; $P = 0.009$, and CA: $t = 4.485$; $P < 0.001$).

3.1.2 Species performance

Incorporating species functional traits into our models, the species survival was mostly affected by traits but without interaction with the planting design (Fig. 2). Specifically, we found that specific leaf area (SLA) was negatively related to species survival ($z = -6.561$; $P < 0.001$; Fig. 2A), whereas wood density (WD: $z = 5.609$; $P < 0.001$; Fig. 2B), and potential height (H: $z = 6.391$; $P < 0.001$; Fig. 2C) were positively related. Only the seed dry mass (SDM) interacted with the planting design ($z = 1.979$; $P < 0.048$; Fig. 2D) to explain species survival, in which larger seed species had higher survival when planted in rows than in clusters. The potential height (H) positively related to the species diameter RGR ($t = 2.67$; $P = 0.007$, Fig. 2E),

irrespective of the planting design. All relationships of the models using the planting systems and species traits are detailed in Online Resource 2.

(Fig. 2)

3.1.3 Cluster performance

Seedling performance at the cluster level was improved when communities were dominated by smaller seed species (low CWM of SDM) but were functionally convergent (low divergence) when using all traits (Fig. 3). Cluster survival increased when the community weighted mean presented low SDM values (z = -2.080; P = 0.038; Fig. 3A) and low functional divergence values (z = -1.965; P = 0.049; Fig. 3B), both independently of fertilization addition. Likewise, clusters with low CWM of SDM decreased the stem biomass RGR, but a stronger negative relationship occurred in non-fertilized clusters (t = 2.930; P = 0.004; Fig. 3C). Moreover, stem biomass RGR was improved when cluster-communities had low values of functional divergence (t = -3.251; P = 0.001; Fig. 3D). All relationships of the models using the fertilization type in cluster communities are in Online Resource 3 (community weighted mean) and Online Resource 4 (diversity indices).

(Fig. 3)

4 Discussion

Our study revealed how an applied nucleation technique influences seedling performance mediated by functional traits at both the species and community (cluster) levels in the early stage of restoration (19 months after planting). Despite higher growth rates were found in rows and higher survivorship in clusters at the individual seedling level, these rates did not differ between designs when including the species functional traits, which were stronger predictors. Interestingly, our findings provide partial support for our first hypothesis. As expected, the slow-growing species, characterized by high SDM, high WD, and low SLA, exhibited higher survival rates. However, contrary to our expectations, neighborhood density did not exert a significant influence on species survivorship, except for species with larger seeds whose survival was improved in row plantations (Fig. 2D). Regarding the species growth rate, functional traits representing fast-growing species had a weak influence on it (only potential height was related to growth). Similarly, our second hypothesis at the cluster level was partially validated. Cluster growth (biomass) increased when it was dominated by highly competitive species (i.e., fast-growing) represented by species with lower seed dry mass. However, unexpectedly, a highly functionally divergent community did not promote cluster survival and growth. All relationships at the cluster level occurred regardless of the addition of fertilizer, except for clusters richer in nutrients dominated by larger-seeded species, which increased their stem biomass storage (Fig. 3C).

Low values of SLA (long leaf lifespan), and high values of H (taller species) and WD (hardwood) mostly related to species survival, regardless of neighborhood density. In general, the lack of difference in species survival between seedlings planted in rows and clusters suggests that biotic interactions did not significantly impact the influence of functional traits on survival, at least in the early restoration phases.

Our results align with the findings of Lasky and colleagues (2014), who did not find a crowding effect in the relationship between species survival and single functional traits. They found that leaf dry matter content (LDMC) and wood density traits were the main factors that explained species survival. Furthermore, plant functional traits associated with a slow life history (large seeds, long-lived leaves, or dense wood) presented a stronger influence on survival than on growth or fecundity (Adler et al. 2014). These species have been associated with greater resistance to drought, making them well-suited for environments characterized by limited water availability (Oliveira et al. 2021). In this regard, slow-growing species present a "slow-safe" strategy, as they are less vulnerable to hydraulic failure through embolism due to their lower photosynthetic rates and fewer stomatal openings (Oliveira et al. 2021). This hydraulic safety strategy should explain the high level of survivorship of slow-growing species in the early stages of restoration, as seedlings face high transpiration rates.

The functional trait of potential height exhibited an intriguing pattern of positively influencing both growth and survival. Species with greater potential heights seemed to gain a competitive advantage in accessing light and, consequently, carbon (Westoby et al. 2002), driving their enhanced growth rates. In addition, height has been associated with a more extensive root system, facilitating access to water during dry periods (Violle et al. 2009), which may further contribute to the survivorship of taller seedlings. Although we found a weak link between SLA and WD and species-relative growth rates, strong relationships have been found in other studies, in a positive and negative direction, respectively (Kunstler et al. 2016; Poorter et al. 2008; Wright et al. 2010). The higher intraspecific plasticity found in seedling leaves (Havrilla et al. 2021) could have influenced the seedling growth rate and clarifies the lack of an SLA effect. In this regard, the SDM trait could be a better indicator of plant strategies to use than SLA, as it has less variation across the ontogenetic stages of plant life history (Adler et al. 2014).

Our study revealed that only SDM exhibited distinct responses in the two planting designs, indicating that this trait could be linked to the presence of biotic interactions within the clusters (Fig. 2D). As expected, larger-seed species demonstrated an improvement in their survival when planted without neighboring seedlings in rows. A larger reserve of seed resources for seedlings after their emergence may explain their higher survival (Moles and Westoby 2004). However, the presence of immediate clustered neighborhoods decreased these species survival. This result suggests that competition, not facilitation (as expected), led to the assemblage of cluster communities. In such cases, larger-seed species could not coexist in the same niche, explained by the principle of limiting functional (MacArthur and Levins 1967). This result at species level may explain the increased seedling performance at cluster level when they were dominated by fast-growing species (low mean values of SDM). This result is likely explained by their high competitiveness and rapid growth due to their high photosynthetic and transpiration rates, typically from species with more acquisitive strategies (Garnier et al. 2016).

A high functional similarity (low FDiv values) in clusters improved seedling performance, for both survival and growth averages. Low FDiv values indicate that the most abundant species exhibit marked similarity and heightened competitiveness, while high FDiv (low functional similarity) values reflect niche differentiation between species in a community (Mouchet et al. 2010). The biotic mechanisms of facilitation or niche differentiation should improve total resource use in a community and increase

productivity (Loreau and Hector 2001) but did not explain seedling performance in such dense cluster communities. Instead, a competitive hierarchy in clusters with a greater abundance of competitive species may exclude less favorable species with functional traits not adapted to this highly competitive community (Chesson 2000), for example, the slow-growing species. Regarding nutrients addition, chemical fertilization improved the cluster biomass stock, but only when they were dominated by slow-growing species (i.e., high SDM values). This result indicates that fertilization may alleviate competition and promote coexistence between those species when soil resources are not limited. Therefore, we rejected our second hypothesis, as we expected that the growth of fast-growing species may be favored in nutrient-rich soil because they are highly competitive (Oliveira et al. 2021).

5 Conclusions

Using plant functional traits, our study offers valuable insights into biotic interactions between seedlings post-plantation, which could optimize restoration strategies. First, plant functional traits can support the process of species selection in restoration, which should be based on a mix of strategies (fast- and slow-growing species) to promote both the survival and growth of seedlings post-plantation. Second, cluster designs that featured dense spacing between seedlings (30 cm) led to a highly competitive community. Despite their overall successful performance, clusters of low functional diversity are not desirable for the long-term recovery of ecosystem functioning. Future research may evaluate the ideal spacing between seedlings in clusters to avoid the dominance of highly competitive species in such aggregated communities. Therefore, restoration practitioners should target facilitation or niche differentiation mechanisms to assemble functionally diverse communities when applying nucleation techniques in restoration.

Declarations

Acknowledgments

6.1 Funding

6.2 Competing Interests

The authors declare that they have no known competing financial and non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6.3 Author Contributions

APM: conceptualization and formal analysis; NTA: data curation and writing - original draft and review & editing; DM: supervision, validation, funding acquisition, and writing - review & editing; AM and MPP: investigation, experimental methodology, and writing - review & editing; JBB: visualization and writing - review & editing. All authors read and approved the final version of the manuscript.

6.4 Data availability

Data will be made available on request.

References

1. Adler P.B., Salguero-Gómez R., Compagnoni A., Hsu J.S., Ray-Mukherjee J., Mbeau-Ache C. and Franco M. 2014. Functional traits explain variation in plant life history strategies. Proceedings of the National Academy of Sciences 111: 740–745. doi:10.1073/pnas.1315179111

2. Alvares C.A., Stape J.L., Sentelhas P.C., Gonçalves J.d.M. and Sparovek G. 2013. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 22: 711–728. https://doi.org/10.1127/0941-2948/2013/0507

3. Bates D., Maechler M., Bolker B., Walker S., Christensen R.H.B., Singmann H., Dai B., Scheipl F. and Grothendieck G. 2009. Package 'lme4'.

4. Bechara F.C., Trentin B.E., Engel V.L., Estevan D.A. and Ticktin T. 2021. Performance and cost of applied nucleation versus high-diversity plantations for tropical forest restoration. Forest Ecology and Management 491: 119088. https://doi.org/10.1016/j.foreco.2021.119088

5. Bertoncello R., Oliveira A.A., Holl K.D., Pansonato M.P. and Martini A.M. 2016. Cluster planting facilitates survival but not growth in early development of restored tropical forest. Basic and Applied Ecology 17: 489–496. https://doi.org/10.1016/j.baae.2016.04.006

6. Callaway R.M., Brooker R.W., Choler P., Kikvidze Z., Lortie C.J., Michalet R., Paolini L., Pugnaire F.I., Newingham B. and Aschehoug E.T. 2002. Positive interactions among alpine plants increase with stress. Nature 417: 844–848. https://doi.org/10.1038/nature00812

7. Carvalho G. 2020. Tools for Interacting with the Brazilian Flora 2020.

8. Charles L.S., Dwyer J.M., Smith T.J., Connors S., Marschner P. and Mayfield M.M. 2018. Species wood density and the location of planted seedlings drive early-stage seedling survival during tropical forest restoration. Journal of Applied Ecology 55: 1009–1018. https://doi.org/10.1111/1365-2664.13031

9. Chave J., Andalo C., Brown S., Cairns M.A., Chambers J.Q., Eamus D., Fölster H., Fromard F., Higuchi N. and Kira T. 2005. Tree allometry and improved estimation of carbon stocks and balance in tropical

forests. Oecologia 145: 87–99. https://doi.org/10.1007/s00442-005-0100-x

10. Chave J., Coomes D., Jansen S., Lewis S.L., Swenson N.G. and Zanne A.E. 2009. Towards a worldwide wood economics spectrum. Ecology letters 12: 351–366. https://doi.org/10.1111/j.1461-0248.2009.01285.x

11. Chesson P. 2000. Mechanisms of maintenance of species diversity. Annual review of ecology and systematics 31: 343–366. https://doi.org/10.1146/annurev.ecolsys.31.1.343

12. Corbin J.D. and Holl K.D. 2012. Applied nucleation as a forest restoration strategy. Forest Ecology and Management 265: 37–46.

13. Flora e Funga do Brasil. 2020. Flora e Funga do Brasil. Jardim Botânico do Rio de Janeiro, Rio de Janeiro.https://floradobrasil.jbrj.gov.br/. Accessed 13 May 2021

14. Fraser L.H. 2020. TRY—A plant trait database of databases. Global change biology 26: 189–190. https://doi.org/10.1111/gcb.14869

15. Gardiner R., Shoo L.P. and Dwyer J.M. 2019. Look to seedling heights, rather than functional traits, to explain survival during extreme heat stress in the early stages of subtropical rainforest restoration. Journal of Applied Ecology 56: 2687–2697. https://doi.org/10.1111/1365-2664.13505

16. Garnier E., Navas M.-L. and Grigulis K. 2016. Plant functional diversity: organism traits, community structure, and ecosystem properties. Oxford University Press.

17. Grossnickle S.C. 2012. Why seedlings survive: influence of plant attributes. New Forests 43: 711–738. https://doi.org/10.1007/s11056-012-9336-6

18. Guimarães Z.T.M., Dos Santos V.A.H.F., Nogueira W.L.P, de Almeida Martins N.O. and Ferreira M.J. 2018. Leaf traits explaining the growth of tree species planted in a Central Amazonian disturbed area. Forest Ecology and Management 430: 618–628. https://doi.org/10.1016/j.foreco.2018.08.048

19. Havrilla C.A., Munson S.M., Yackulic E.O. and Butterfield B.J. 2021. Ontogenetic trait shifts: Seedlings display high trait variability during early stages of development. Functional Ecology 35: 2409–2423. https://doi.org/10.1111/1365-2435.13897

20. Holl K.D., Reid J.L., Cole R.J., Oviedo-Brenes F., Rosales J.A. and Zahawi R.A. 2020. Applied nucleation facilitates tropical forest recovery: Lessons learned from a 15-year study. Journal of Applied Ecology 57: 2316–2328. https://doi.org/10.1111/1365-2664.13684

21. Holl K.D., Zahawi R.A., Cole R.J., Ostertag R. and Cordell S. 2011. Planting Seedlings in Tree Islands Versus Plantations as a Large-Scale Tropical Forest Restoration Strategy. Restoration Ecology 19: 470–479. https://doi.org/10.1111/j.1526-100X.2010.00674.x

22. Kattge J., Bönisch G., Díaz S., Lavorel S., Prentice I.C., Leadley P., Tautenhahn S., Werner G.D., Aakala T. and Abedi M. 2020. TRY plant trait database–enhanced coverage and open access. Global change biology 26: 119–188. https://doi.org/10.1111/gcb.14904

23. Kunstler G., Falster D., Coomes D.A., Hui F., Kooyman R.M., Laughlin D.C., Poorter L., Vanderwel M., Vieilledent G. and Wright S.J. 2016. Plant functional traits have globally consistent effects on competition. Nature 529: 204–207. https://doi.org/10.1038/nature16476

24. Laliberté E., Legendre P., Shipley B. and Laliberté M.E. 2014. Package 'fd'. Measuring functional diversity from multiple traits, and other tools for functional ecology, pp. 0–12.

25. Lasky J.R., Uriarte M., Boukili V.K. and Chazdon R.L. 2014. Trait-mediated assembly processes predict successional changes in community diversity of tropical forests. Proceedings of the National Academy of Sciences 111: 5616–5621. https://doi.org/10.1073/pnas.1319342111

26. Laughlin D.C., Gremer J.R., Adler P.B., Mitchell R.M. and Moore M.M. 2020. The net effect of functional traits on fitness. Trends in ecology & evolution 35: 1037–1047. https://doi.org/10.1016/j.tree.2020.07.010

27. Loreau M. and Hector A. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412: 72–76. https://doi.org/10.1038/35083573

28. MacArthur R. and Levins R. 1967. The limiting similarity, convergence, and divergence of coexisting species. The american naturalist 101: 377–385. https://doi.org/10.1086/282505

29. Maestre F.T., Callaway R.M., Valladares F. and Lortie C.J. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of ecology 97: 199–205. https://doi.org/10.1111/j.1365-2745.2008.01476.x

30. Martínez-Garza C., Bongers F. and Poorter L. 2013. Are functional traits good predictors of species performance in restoration plantings in tropical abandoned pastures? Forest Ecology and Management 303: 35–45. https://doi.org/10.1016/j.foreco.2013.03.046

31. Mayfield M.M. and Levine J.M. 2010. Opposing effects of competitive exclusion on the phylogenetic structure of communities. Ecology letters 13: 1085–1093. https://doi.org/10.1111/j.1461-0248.2010.01509.x

32. Moles A.T. and Westoby M. 2004. Seedling survival and seed size: a synthesis of the literature. Journal of ecology 92: 372–383. https://doi.org/10.1111/j.0022-0477.2004.00884.x

33. Mouchet M.A., Villéger S., Mason N.W. and Mouillot D. 2010. Functional diversity measures: an overview of their redundancy and their ability to discriminate community assembly rules. Functional Ecology 24: 867–876. https://doi.org/10.1111/j.1365-2435.2010.01695.x

34. Oliveira-Filho A. 2017. NeoTropTree: Tree flora of the neotropical region: a database involving biogeography, diversity and conservation. Universidade Federal de Minas Gerais. http://www.neotroptree.info. Accessed 21 July 2023

35. Oliveira R.S., Eller C.B., Barros F.d.V., Hirota M., Brum M. and Bittencourt P. 2021. Linking plant hydraulics and the fast–slow continuum to understand resilience to drought in tropical ecosystems. New Phytologist 230: 904–923. https://doi.org/10.1111/nph.17266

36. Perez-Harguindeguy N., Diaz S., Garnier E., Lavorel S., Poorter H., Jaureguiberry P., Bret-Harte M.S., Cornwell W.K., Craine J.M. and Gurvich D.E. 2016. Corrigendum to: New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of botany 64: 715–716. https://doi.org/10.1071/BT12225_CO

37. Pommerening A. and Muszta A. 2016. Relative plant growth revisited: Towards a mathematical standardisation of separate approaches. Ecological Modelling 320: 383–392.

https://doi.org/10.1016/j.ecolmodel.2015.10.015

38. Poorter H., Lambers H. and Evans J.R. 2014. Trait correlation networks: a whole-plant perspective on the recently criticized leaf economic spectrum. New Phytologist 201: 378–382. https://www.jstor.org/stable/newphytologist.201.2.378

39. Poorter L., Wright S.J., Paz H., Ackerly D.D., Condit R., Ibarra-Manríquez G., Harms K.E., Licona J., Martinez-Ramos M. and Mazer S. 2008. Are functional traits good predictors of demographic rates? Evidence from five neotropical forests. Ecology 89: 1908–1920. https://doi.org/10.1890/07-0207.1

40. Rasband W. 2012. ImageJ: Image processing and analysis in Java. Astrophysics Source Code Library, p. ascl: 1206.1013.

41. Silliman B.R., Schrack E., He Q., Cope R., Santoni A., van der Heide T., Jacobi R., Jacobi M. and van de Koppel J. 2015. Facilitation shifts paradigms and can amplify coastal restoration efforts. Proceedings of the National Academy of Sciences 112: 14295–14300. https://doi.org/10.1073/pnas.1515297112

42. Team R.C. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

43. Toledo-Aceves T., Bonilla-Moheno M., Sosa V.J., López-Barrera F. and Williams-Linera G. 2022. Leaf functional traits predict shade tolerant tree performance in cloud forest restoration plantings. Journal of Applied Ecology 59: 2274–2286. https://doi.org/10.1111/1365-2664.14128

44. Trinder C.J., Brooker R.W. and Robinson D. 2013. Plant ecology's guilty little secret: understanding the dynamics of plant competition. Functional Ecology 27: 918–929. https://doi.org/10.1111/1365-2435.12078

45. Vieira B.C., Ferreira F.S. and Gomes M.C.V. 2015. Physical and hydrologial soil properties and the shallow landslides in the serra do mar paulista/propriedades fisicas e hidrologicas dos solos e os escorregamentos rasos na serra do mar paulista. Ra'e Ga: 269–288.

46. Violle C., Garnier E., Lecoeur J., Roumet C., Podeur C., Blanchard A. and Navas M.-L. 2009. Competition, traits and resource depletion in plant communities. Oecologia 160: 747–755. https://doi.org/10.1007/s00442-009-1333-x

47. Werden L.K., Alvarado J. P., Zarges S., Calderón M. E., Schilling E.M., Gutiérrez L. M. and Powers J.S. 2018. Using soil amendments and plant functional traits to select native tropical dry forest species for the restoration of degraded Vertisols. Journal of Applied Ecology 55: 1019–1028. https://doi.org/10.1111/1365-2664.12998

48. Westoby M., Falster D.S., Moles A.T., Vesk P.A. and Wright I.J. 2002. Plant ecological strategies: some leading dimensions of variation between species. Annual review of ecology and systematics 33: 125–159. https://doi.org/10.1146/annurev.ecolsys.33.010802.150452

49. Wright I.J., Reich P.B., Westoby M., Ackerly D.D., Baruch Z., Bongers F., Cavender-Bares J., Chapin T., Cornelissen J.H. and Diemer M. 2004. The worldwide leaf economics spectrum. Nature 428: 821–827. https://doi.org/10.1016/j.tree.2010.11.011

50. Wright S.J., Kitajima K., Kraft N.J., Reich P.B., Wright I.J., Bunker D.E., Condit R., Dalling J.W., Davies S.J. and Díaz S. 2010. Functional traits and the growth–mortality trade-off in tropical trees. Ecology

91: 3664–3674. https://doi.org/10.1890/09-2335.1

51. Yarranton G. and Morrison R. 1974. Spatial dynamics of a primary succession: nucleation. The Journal of Ecology: 417–428. https://doi.org/10.2307/2258988

52. Zimback L.B., Prado P.I., Pansonato M.P., Franco G.A. and Martini A.M. 2023. Strong spatial and temporal limitations in seed arrival as complementary mechanisms for species coexistence in a tropical Atlantic coastal forest. Plant Ecology 224: 267–281. https://doi.org/10.1007/s11258-023-01294-5
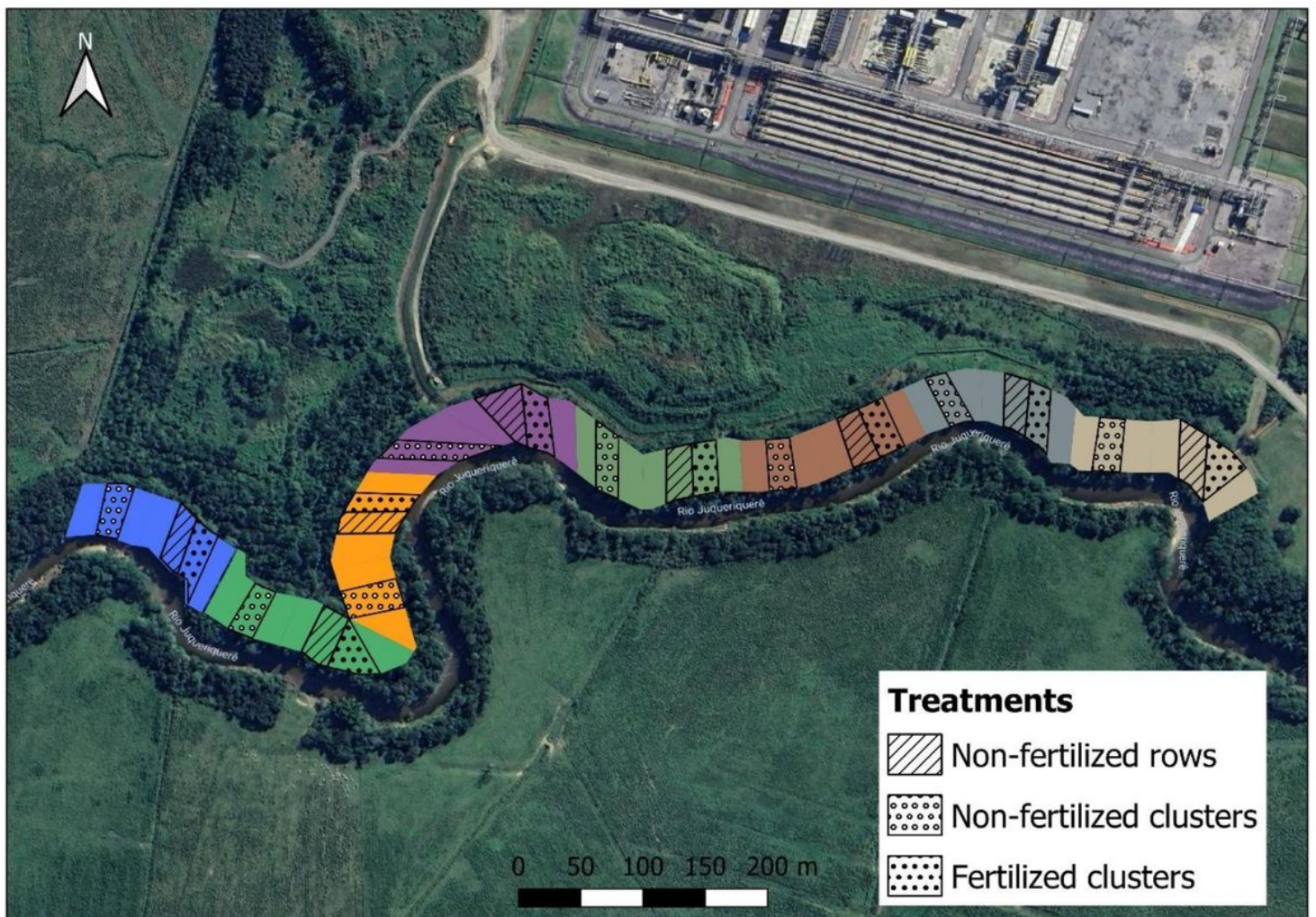
Figures

Figure 1

Experiment design of ecological restoration in a riparian tropical forest locate at Caraguatatuba municipality, São Paulo, Brazil. The experimental site was divided into eight blocks (distinct colors in the map) that contained one replicate for each treatment of planting design (rows and clusters) and fertilization. We marked only blocks used in this study (non-fertilized rows and clusters, and fertilized

clusters). The figure was done using QGIS version 3.28.15. Satellite image from Google, Image © 2021 Maxar Technologies
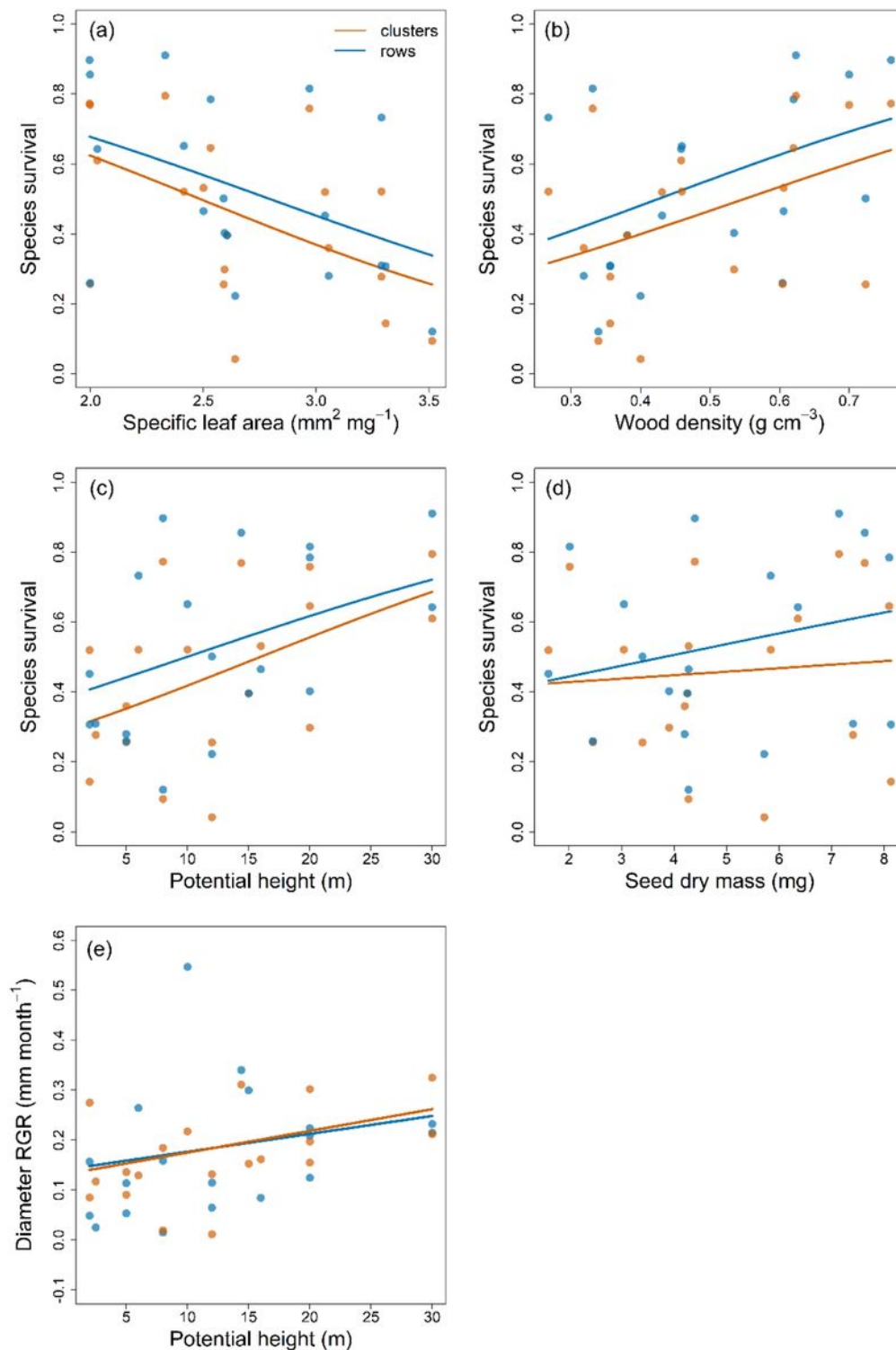

Figure 2

Significant relationships between the average of the species performance with their mean functional trait in two distinctive planting designs (rows vs. clusters). Percentage of species survival as a function of (A) specific leaf area (log-scale), (B) woody density (g.cm^{-3}); (C) potential height (m); (D) seed dry mass (log-

scale). Species relative growth rate (RGR) in diameter at ground level (DGL) as a function of (E) potential height (m)
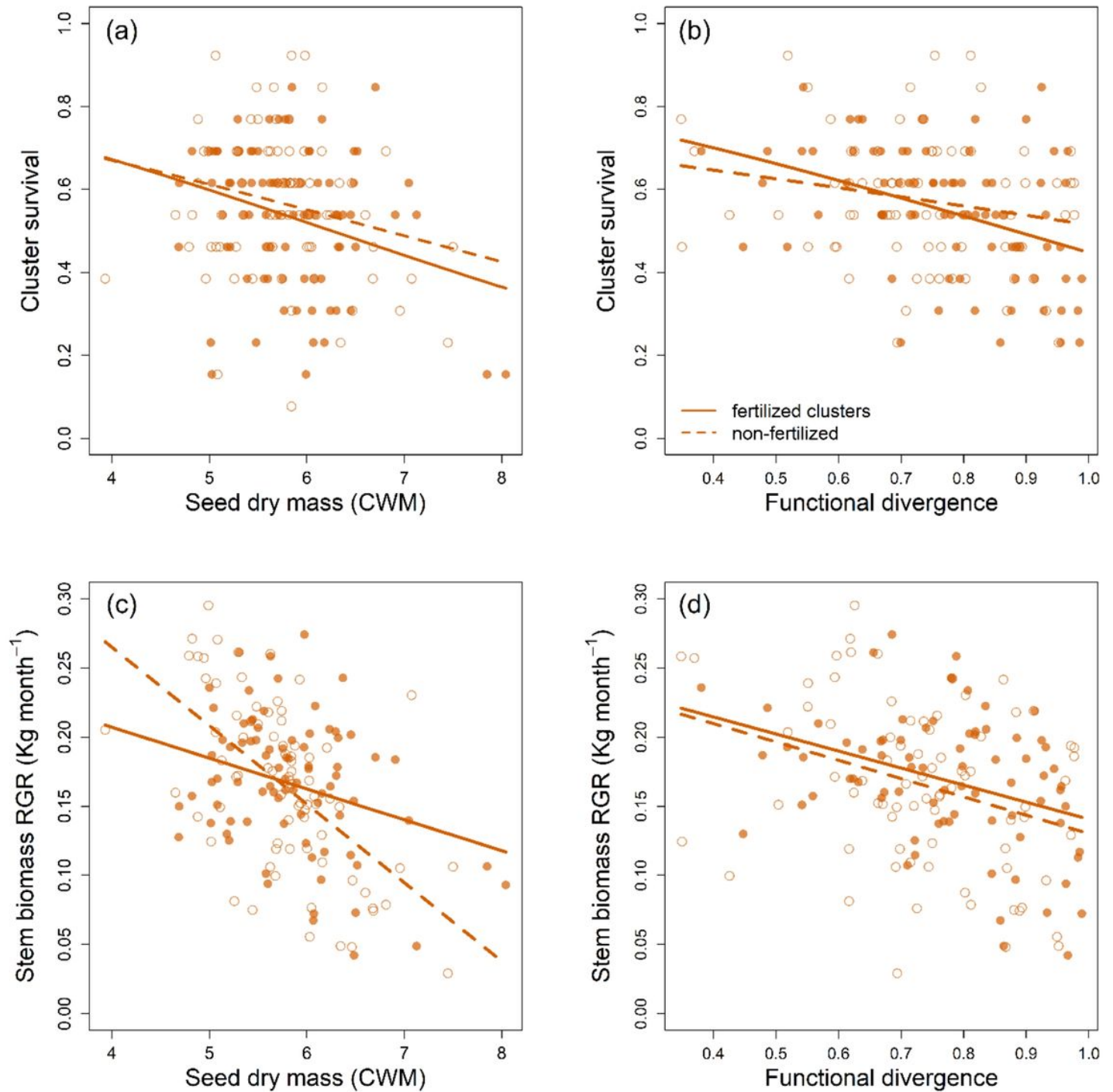

Figure 3

Relationships between the cluster performance in two fertilization types (fertilized and non-fertilized clusters) with their trait community-weighted mean (CWM). Cluster survival with (A) CWM of seed dry mass (log-scale), (B) functional divergence. Stem biomass relative growth rate (RGR) with (C) CWM of seed dry mass (log-scale) and (D) functional divergence

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ESM1.xlsx
- ESM2.xlsx
- ESM3.xlsx
- ESM4.xlsx