# Seedlings and soil seed bank diversity and composition of a secondary and old tropical mountain forest in Colombia.

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# Seedlings and soil seed bank diversity and composition of a secondary and old tropical mountain forest in Colombia.

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# 1. Abstract.

**Introduction.** Soil seed banks (SSBs) are essential in ecological restoration since they reflect the composition of a local community, propagule dispersal, and they act as seed stocks. SSBs are also important determinants of seedling emergence and establishment. Many mountain forests near Bogota (Colombia) have been regenerating and, although there are studies regarding the standing vegetation in this area, few have focused on SSBs and seedlings. Thus, understanding SSB and seedling communities would provide valuable information about the dynamics of regeneration in these forests.

**Objective and hypothesis.** My objective was to understand how SSBs and seedling (Sdl) diversity changed between young (SF) and old (OF) Tropical Mountain Forest and how soil characteristics can play a role in determining SSBs and Sdl composition. Despite marked differences in species composition, forest structure and edaphic conditions between SF and OF, I expected to find similarities in species composition in the SSBs between young and old forests. That is because SSBs are dominated by small seeded early-successional forests For the seedling's community, we expected to find differences in composition among the old and young forest.

**Materials and Methods.** Ten plots were established in three different sites around Bogota as part of the Rastrojos project, five from OF and five from SF. I evaluated the SSB using the seedling emergence method in a greenhouse and conducted seedling surveys in the field to evaluate the seedling community. Also, I used nutrient fluxes and nutrient concentration data from the database of the Rastrojo project to analyze how SSB related to edaphic conditions.

**Results and Discussion.** I found a Bray Curtis index of 0.48 between OF SSB and SF SSB, and a value of 0.51 between SF Sdl and OF Sdl. These findings suggest that the community composition of either SSB or Sdl do not differ largely between the types of forest. For the SSBs, I found that plots that were more similar regarding pH or NH4-N flux, were also more similar regarding SSB community composition. These edaphic characteristics are likely to affect seed viability and germination capacity. In contrast, for the seedling’s community, none of the other soil variables (bulk density, aluminum flux, phosphorus flux, nitrites flux, nitrates flux, C%, N%), were related to community composition except for soil and air temperature,

**Conclusion.** Although SSBs are important in the early stages of regeneration, SSBs are partially different between successional stages. Likewise, seedlings community composition is not that different between SF and OF. Therefore, other factors (pH, NH4-N, soil temperature) may be more critical for the species' establishment in mature forests. Also, the site of study plays a major role for seedling and SSBs community assembly. Besides, according to the life stage (seed or seedling) different environmental factors are important in community assembly. It is necessary to continue studying these ecosystems since little is known about them, and furthering our knowledge is essential to understanding their ecology and improve management programs.

**Keywords**: ecological succession, seeds, environmental factors, germination.

## 2. Introduction

Tropical Andean mountains ecosystems have extreme species richness due to their elevational gradients and complex climatic characteristics (Rahbek et al., 2019). They are home to at least 20,000 species of endemic plants (Myers et al., 2000), representing 6.7% of all plant species worldwide. In addition, they harbor 5.7% of endemic vertebrates (Myers et al., 2000). Nevertheless, a significant portion of the Andes tropical mountains forests (ATMF) have been occupied by agro-pastoral and development activities (Rubiano et al., 2017) such as farming, mining, and human settlements, and for centuries these activities have caused ecosystem transformation and degradation. In fact, in the Eastern Cordillera of the Colombian Andes, including the Sabana of Bogota, about 20% of the original forests remain (Etter & Villa, 2000). However, several of these disturbed areas have been regenerating naturally in the last few years due to human migrations (Rubiano et al., 2017), and secondary forests have been established (Chazdon et al., 2016). Despite ATMFs' ecological and social importance, little is still known about the ones near Bogotá. Recent studies have been done on floristic composition (Hurtado-M et al., 2021) and changes and dynamics of the secondary forest through time (Calbi et al., 2020).

The regeneration and establishment of secondary forests (SF) represent a low-cost strategy for carbon sequestration (Chazdon et al., 2016). In addition, SF serves as a habitat for many species

(Chazdon, 2008) and provides many ecosystems services (Etter & Villa, 2000). Thus, conserving these ecosystems is advantageous to reduce climate change and prevent biodiversity loss. Therefore, it is essential to understand the dynamics of succession in tropical secondary forests and promote conservation and natural regeneration (Rubiano et al., 2017).

Chazdon (2008) describes secondary succession as a “long-term directional change in community composition following a disturbance event, often at a large spatial unit.” Forest succession is generally characterized by replacing fast-growing, shade-intolerant species with shade-tolerant and slower-growing organisms. As the canopy starts to close, the abundance of shade-intolerant plant species declines (Chazdon, 2017). However, despite knowing the generality of succession, this process is still difficult to predict because its trajectory depends on land use before the succession, soil fertility, and climate variables (Chazdon, 2008). Also, environmental factors, landscape features (distance between mature forest and fragments of SF), and seed dispersal dynamics affect the composition of the community (Chazdon, 2008).

On the other hand, tropical old growth forests are characterized by having multilayered canopies with some gaps and exhibit great abundance of shade-tolerant and slow-growing species (Hilbert & Wiensczyk, 2007). Therefore, to recognize different stages of succession or to compare young and old forests, it is essential to analyze the community composition of adults, juveniles, and seedlings, as well as the soil seed banks (SSBs). SSBs are the result of the site's composition and the diversity of the overstory, and it is fundamental to understand the vegetation dynamics before succession (Ma et al., 2009). In addition, SSBs are determined by the current dispersal of propagules from the local community and more distant sources. Ma et al. (2009) proposed that SSBs play an essential role in ecological restoration as many lost target species could be reestablished from it. Hence SSBs facilitate population persistence (Pakeman et al., 2012), which means that they facilitate the permanence of critical species in future scenarios, allowing them to maintain an ecosystem’s identity. Besides, SSBs play an essential role in early successional stages because they provide the seeds and seedlings of pioneers to initiate the successional process (Ma et al., 2018).

Studying the SSB and comparing it to the standing vegetation helps clarify to what extent forest regeneration relies on previously accumulated seeds or the dispersal of new propagules (Pakeman et al., 2012). Nonetheless, most successional studies in tropical forests have focused on tree adult communities, leaving aside the dynamics of SSBs but also seedlings composition (Chazdon, 2008), thus limiting our understanding of the ecology of secondary forests.

Few studies have been done on SSBs and seedling composition in Andean secondary forests. For instance, Gelviz-Gelvez, Sánchez, et al., (2016) analyzed different methods, such as direct separation and germination, for assessing the composition of the soil SSB. This study was conducted in Colombia, and they found differences between the SSB of shrublands (early successional stage) and old-growth forests (late successional stage). Also, in the Bolivian Andes, some studies have been made regarding the effects of different degrees of disturbance on SSBs (Lippok et al., 2013). However, little is known about species richness in SSBs and how they relate to forest successional status, but also how they are influenced by soil nutrients and pH. Thus, it is crucial to study how SSB could be related to soil properties since the Andean tropics are characterized by complex topography and variation in climate and soil, that ultimately can have large effects on the community composition (Rahbek et al., 2019); besides understanding these ecosystems can promote the development of better conservative and management programs.

The objective of this study was to understand a) how SSBs and seedling diversity change among young (SF) and old (OF) Andean Tropical Mountain Forest and, b) how they relate to soil fertility and air temperature (12 cm from soil surface). I characterized and compared the diversity and composition of the soil seed bank in these two types of forest. I expected to find significant similarity in the community composition in the SSB between young and old forests, because the composition of the soil seed bank is dominated by persistent seeds, which can live more than a year buried in the soil (Dalling & Brown, 2009) and are less likely to be predated on or suffer damage by soil pathogens (Daïnou et al., 2011). These types of seeds are characteristic of pioneer species with high colonization rates, and high seed production and dispersal rates  (Dalling & Brown, 2009). In contrast, old forest species tend to produce large seeds that do not remain for long in the SSB due to their constant moistness (Daïnou et al., 2011), which means that they do not stay viable in the soil for more than a year. In addition, I assessed the community of seedlings among young and old forests. Yet, and in contrast to the SSBs, I expected to find differences in community composition because seedlings of shade-tolerant species can successfully establish in the dark understory of mature forests, while in young forests fast-growing pioneers seedlings can grow successfully due to higher resource availability (Chazdon, 2017).

## 3. Materials and methods:

### 1. Study sites:

The study was conducted in three different geographical sites near Bogota, Colombia: Torca, Tabio, and Guasca, at altitudes between 2600 and 3200 meters (Fig. 1). These sites are characterized by average annual precipitation of 600 to 1200 mm and two rainy seasons (April-June and September-November) (Hurtado-M et al., 2021). They include upper Andean tropical mountain forests in different stages of succession. The first one (Torca) is in the Eastern hills of Bogota and it is part of the Reserva Forestal Protectora del Río Bogotá and the Área de Reserva Forestal Regional of Bogota (Minambiente, 2016). The second site (Tabio) is private that was mostly used for agro-pastoral activities. It is composed of an extensive secondary forest and a few old-growth forest patches. The last place (Guasca) is in a civil society private reserve (Encenillo Biological Reserve) managed by the Natura Foundation (Fig. 1) which had limestone mining activity until the late 1990s (Hurtado-M et al., 2021).

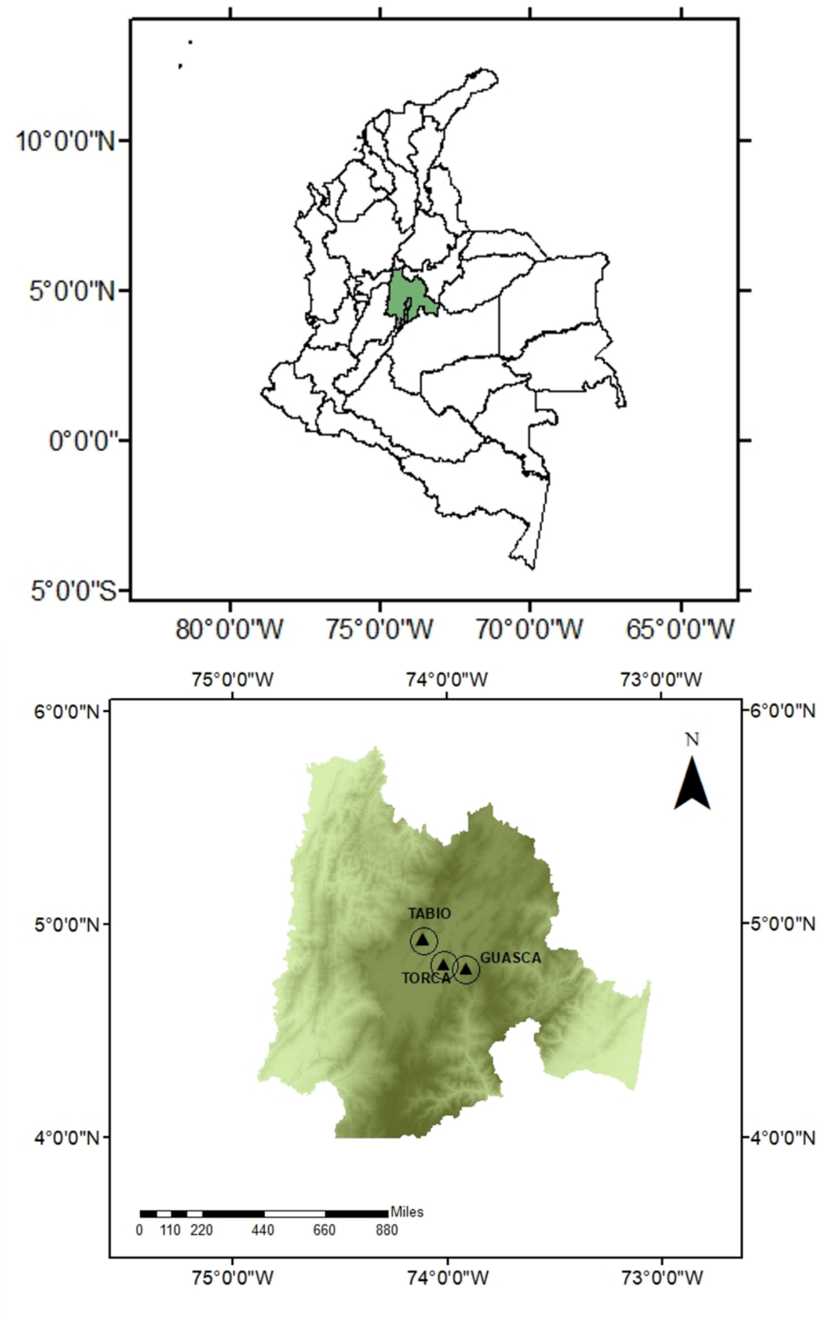

**Fig. 1** Map of the study sites. Top: each triangle shows a study site (Tabio, Torca, Guasca). Bottom: Colombia's map. The Green zone is Cundinamarca and Bogota, where the study was conducted.

Ten 20 m x 20 m permanent plots were established at the three sites as part of the "Rastrojos project". The project aim is to study the ecology of ATMF near Bogota with a network of 38 plots in collaboration with researchers from “Universidad del Rosario” and “Instituto Alexander von Humboldt”. The age of SF plots varied between 10 and 30 years and they were characterized by low canopy height (Hurtado-M et al., 2021). In contrast, the age of OF plots was around 60 to 100 years old and they were characterized by a multilayered canopy (Hurtado-M et al., 2021). In Tabio and Guasca, eight plots were established: four in each site where two represented SF and the other two OF. In Torca, two plots were established, one for SF and the other for the OF. Also, four subplots of 2m x 2m were established in each plot to do the seedlings survey (Fig. 2).

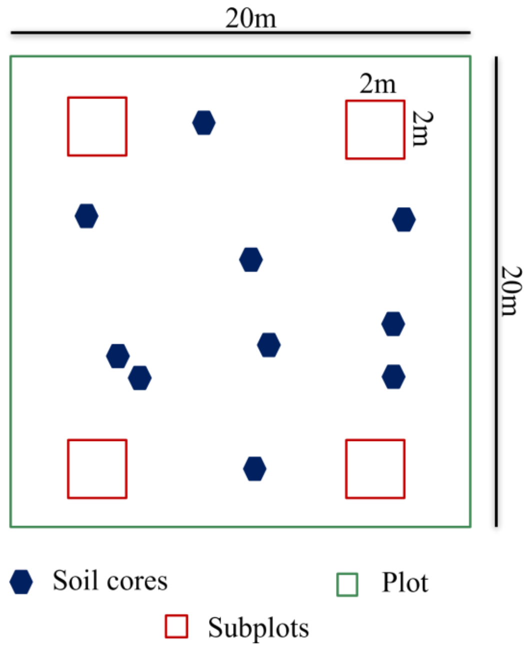

**Fig. 2** Arrangement of the study plots, subplots, and soil samples. Plots are represented by green squares, subplots by red squares and soil cores are represented by blue hexagons. In Guasca we have a total of four plots where plot number 3 and 5 (P3 and P5) are from OF while P4 and P6 are from SF (16 subplots and 40 soil cores). In Tabio we have four plots where P9 and P10 are from OF and P7 and P8 are from SF (16 subplots, 40 soil cores). At Torca we have two plots where P12 is from OF and P14 is from SF (8 subplots and 20 soil cores).

## 2. *Seed bank sampling and germination*

Ten soil cores were extracted per plot in December 2021, at the end of the rainy season, since seed density is in theory, higher at that time (Abreu et al., 2021). Cores were extracted randomly at each of the ten plots to assess seedling emergence from the SSB. Extracting 10 soil cores at random in each plot was necessary if we wanted to have a complete seed identity of the plot since, at different points in the quadrant, different species can be found. For example, the richness of small-seeded species can be greater at the plot forest margins meanwhile at the plot center other species may be found (Lippok et al., 2013). The cores were of 4.45 $dm^3$. Soil cores were transported to a greenhouse to quantify germinating seeds. Soil was placed in trays and all roots, litter, and tuber were removed by hand; each soil core was placed on top of a mixture of 20% peat, 50% black soil and 30% rice husk. With the substrate, each soil sample was ≤ 5cm in depth.

I watered the samples 30 seconds daily with a drip irrigation system to keep them moist (Yang & Li, 2013). Afterward, seed quantification and identification were made using the seedling emergence method proposed by Ter Heerdt et al. (1996), since seed separation methods are not appropriate for identifying small seeds. Given that recognizing seeds is complicated, I waited until they germinated and produced two real leaves to identify them as morphospecies. With this method, I counted the germinated plants every two weeks approximately and performed a total of ten counts, corresponding to 131 days of germination. Also, I removed seedlings from the trays as soon as they had at least two real leaves. I then transplanted the individuals to other trays, where they continued growing until recognition could be done (Ter Heerdt et al., 1996). However, I was only able to determine the taxonomic identity of six morphotypes out of 62 morphotypes. Additionally, I calculated seedling density by dividing the number of germinated seeds by the volume of each sample. Finally, we tracked germination for five months.

### **3.** *Seedling survey*

In each subplot (2m x 2m – 40 subplots), I measured, counted, and identified tree and shrub seedlings that were at least 5 cm height using rapid color guides of the vegetation of the study sites, a book about the vegetation of the zone produced by the “Corporación Autónoma Regional de Cundinamarca” (CAR) (Mahecha Vega & Corporación Autónoma Regional de Cundinamarca., 2004) and the help of some researchers such as Ana Belén Hurtado and Carlos Vargas. We were able to determine the taxonomic identity of 44 morphotypes out of 97 morphotypes.

### **4.** *Soil pH:*

The pH of the soil core samples (100 samples - 10 from each plot) was obtained by measuring, with a digital pH meter, a mixture of 1:10 soil/deionized water. Although a mixture of 1:5 soil/water is commonly used, we did not have enough soil to apply this method. Using a mixture of 4 g of soil with 40 mL of deionized water we measured the pH at 20 °C (Long et al., 2018).

### **5.** *Nutrient fluxes and soil characteristics:*

Nutrient data for the plots was obtained from a database of the Rastrojo' s project. These data were obtained using the Plant Root Simulator (PRS) and ten were installed in each plot. I analyzed their data on nutrient fluxes (NH4-N, NO3-N, P, Al in ug/10cm/30days), the total content of C%, N%, at 0-15 cm deep, and bulk density (g/cm3) that was obtained at 15-30 cm deep. In addition, temperatures at 6 and 12 cm depth were obtained every 15 minutes for four months, with the TMS-4 datalogger from TOMST.

### **6.** *Data and statistical analysis:*

I conducted the statistical analyses in RStudio. Since I did not conducted a seed separation method where I could evaluate the SSB directly through the seeds, I calculated seed density as the number of germinated seeds by the volume of the soil core. I conducted a Mann-Whitney U test (R package: CAR; Fox, 2021) to identify differences in seed density between the SSBs of SF and OF. I also performed a Kruskal-Wallis test (Package in R: CAR; Fox, 2021) to look for significant differences between the ten plots and the three study sites. Then, I evaluated the diversity of the SSB and seedlings of the two types of forests using Hill numbers (richness, Shannon index, and Inverse Simpson's index) (Package in R: hillR; Chao et al., 2014). I conducted a two-way ANOVA (Package in R: CAR; Fox, 2021) to identify differences in diversity between SF and OF at the different sites. Then, I conducted a Tukey test to identify differences between individual. Next, I applied the Bray Curtis index (Package in R: vegan; Oksanen et al., 2020) to compare similarityies in species composition of seedlings and soil seed banks. Finally, a Non-metric Multidimensional Scaling (NMDS) (Package in R: vegan; Oksanen et al., 2020) was used to examine the relationship between the SSBs of the plots, the seedlings, and soil variables such as pH, bulk density, aluminum flux, phosphorus flux, nitrites flux, nitrates flux, C%, N% and soil temperature (Yang & Li, 2013). Finally, I tested the correlation between soil factors and community assemblage with the Mantel Test and ANOSIM Test (R package: ade4; Dray & Dufour, 2007)

## 4. Results

### ***Soil Seed Bank:***

### *A.* Seed density, richness, and diversity:

I identified a total of 62 morphotypes in de SSB after five months of germination (13/12/2021 – 06/05/2022); 24 species only belonged to old forest plots, 16 only to secondary forest plots, and 22 were shared (Table 1). The site with the highest morphotypes was Tabio (43 in total), especially in the old forest samples. In contrast, Torca had the lowest number of morphotypes (10 in total). Only one morphotype (number 43; *Myrsine*) was shared in the two types of forest in all three sites.

**Table 1.** Number of morphotypes germinated in the soil seed banks (SSB) of the three sampling sites.

| Site | Secondary forest (SF) | Old forest (OF) | Shared morphotypes (SF & OF) | Total |
|---|---|---|---|---|
| **SSB – three sites** | 38 | 46 | 22 | 62 |
| **Guasca** | 21 | 21 | 10 | 32 |
| **Tabio** | 26 | 32 | 15 | 43 |
| **Torca** | 8 | 3 | 1 | 10 |

I did not find significant differences in seed density between the two types of forests (W = 1033; p-value = 0.1334; Table 2; Table 7A Supplementary material). Yet, significant differences in seed density between sites ($x^2$ = 22.079; p-value = 1.605e-05) and plots ($x^2$ = 45.46; p-value = 7.588e-07) (Table 2) were found. Since some of the significant differences were between plots of different sites (Table 3A. Supplementary material), I did not focus on them. Nevertheless, I found significant differences between Guasca plots, specifically between P3 OF and P6 SF, and between P3 OF and P4 SF (Fig. 3; Table 3A. Supplementary material). Torca had the lowest number of morphotypes and seed density which means a lower germination activity.

**Table. 2** Comparisons in terms of seed density. Where p >0.05 and "*" represents significant differences.

| Comparisons | Kruskal Wallis (p-value) |
|---|---|
| **Old forest vs. Secondary Forest** | 0.1334 |
| **Between plots** | 7.588e-07* |
| **Between sites** | 1.605e-05* |

Regarding diversity indexes, I did not find significant differences between the SSB of the two types of forests in any of the calculated indexes (p > 0.05; Table 3). In terms of richness, OF SSB had a slightly greater mean value (14.4 ± 8.5 – Table 3) compared to the SF SSB of (13.6 ± 5.81), but the differences were not significant. The SF SSB exhibited a greater value of the Inverse Simpson index (5.90 ± 4.52) than OF SSB (4.77+ 2.38), but again the differences were not significant. In addition, OF SSB and SF SSB had similar Shannon index (Table 3). Finally, I did not obtain any significant differences in the three Hill numbers for the two-way ANOVA used to evaluate the site and type of forest (richness, Shannon index, Inverse Simpson index) (Table 4A – Supplementary material).

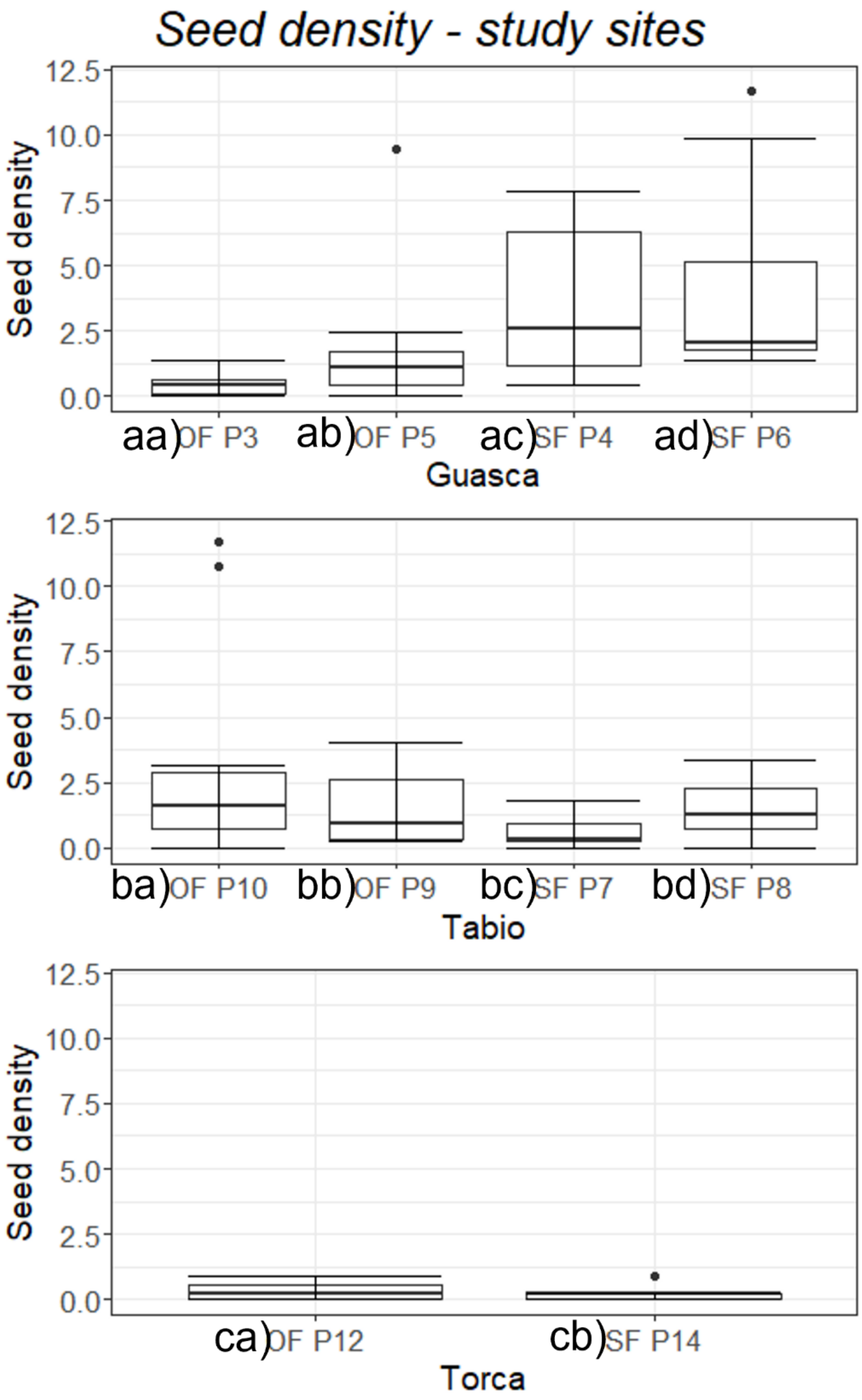

**Fig. 3.** Box plot of seed density in the soil seed bank of the three sites. We found significant differences between: “aa” and “ad”,“aa” and “ac”, “ba” and “cb”, “ad” and “bc”, “ac” and “ca”, “ac” and “cb”, “ad” and “ca”, “ad” and “cb” (Table 3A Supplementary material). Beside we found significant differences

between these sites: Tabio and Torca (p-value = 3.54e-04; Table 3A Supplementary material), Guasca and Torca (p-value = 1.43e-05; Table 3A Supplementary material)

**Table 3.** Hill numbers. According to the q parameter, a value was calculated for each plot, obtaining five values for each type of forest (SF or OF) and class (SSB or Sdl). With those values, we obtained the mean for each group of study.

| | | **Sdl OF** | **Sdl SF** | **SSB OF** | **SSB SF** | **P-value (t-test)** |
|---|---|---|---|---|---|---|
| **n** | | 5 | 5 | 5 | 5 | |
| **Richness (q=0)** | mean | 18.8 ± 5.67 | 17.6 ± 7.89 | 14.4± 8.5 | 13.6 ± 5.8 | |
| | Sdl OF – Sdl SF (t = -0.28) | | | | | 0.79 |
| | SSB OF – SSB SF (t = -0.17) | | | | | 0.87 |
| **Shannon index (q=1)** | mean | 9.63 ± 1.99 | 9.24 ± 3.73 | 7.03 ± 2.98 | 7.57 ± 5.64 | |
| | Sdl OF – Sdl SF (t = -0.20) | | | | | 0.85 |
| | SSB OF – SSB SF (t = 0.19) | | | | | 0.85 |
| **Inverse Simpson index (q=2)** | mean | 6.14 ± 1.62 | 6.06 ± 2.84 | 4.77± 2.38 | 5.9 ± 4.52 | |
| | Sdl OF – Sdl SF (t = -0.05) | | | | | 0.96 |
| | SSB OF – SSB SF (t =0.49) | | | | | 0.64 |

### B. SSB community composition and soil factors:

There were multiple germination peaks (Fig. 4) at different times, depending on the forest type. In OF, I evidenced two germination peaks: the first on the 61st day (22.69% of the total germination in OF) and the second on the 131st day (23% of the total germination in OF). Although I did not observe a marked peak of gemination in SF, 34.29% of the total germination in SF occurred earlier than in OF (between days 33 and 47). It is important to note that Figure 4 did not consider the germination data of morphotype 26 (*Ulex europaeus* – commonly call "gorse"), which had a germination peak in SF samples in Guasca on day 33 (152 individuals; Fig. 2AA - Supplementary material). Although this invasive species was important in the SBB of Guasca, we decided to exclude it and focus on the native species in these two forest types. Also, there were differences in the composition of the SSB between SF and OF (Bray-Curtis index = 0.48) (Fig. 4). Some morphotypes were only present in the mature forests, while others were found in both types of forests, but in different quantities. Additionally, I found that SSB by sites did not clearly separated between forest type or sites, except for Guasca that clustered in three out of four plots (Fig. 5).

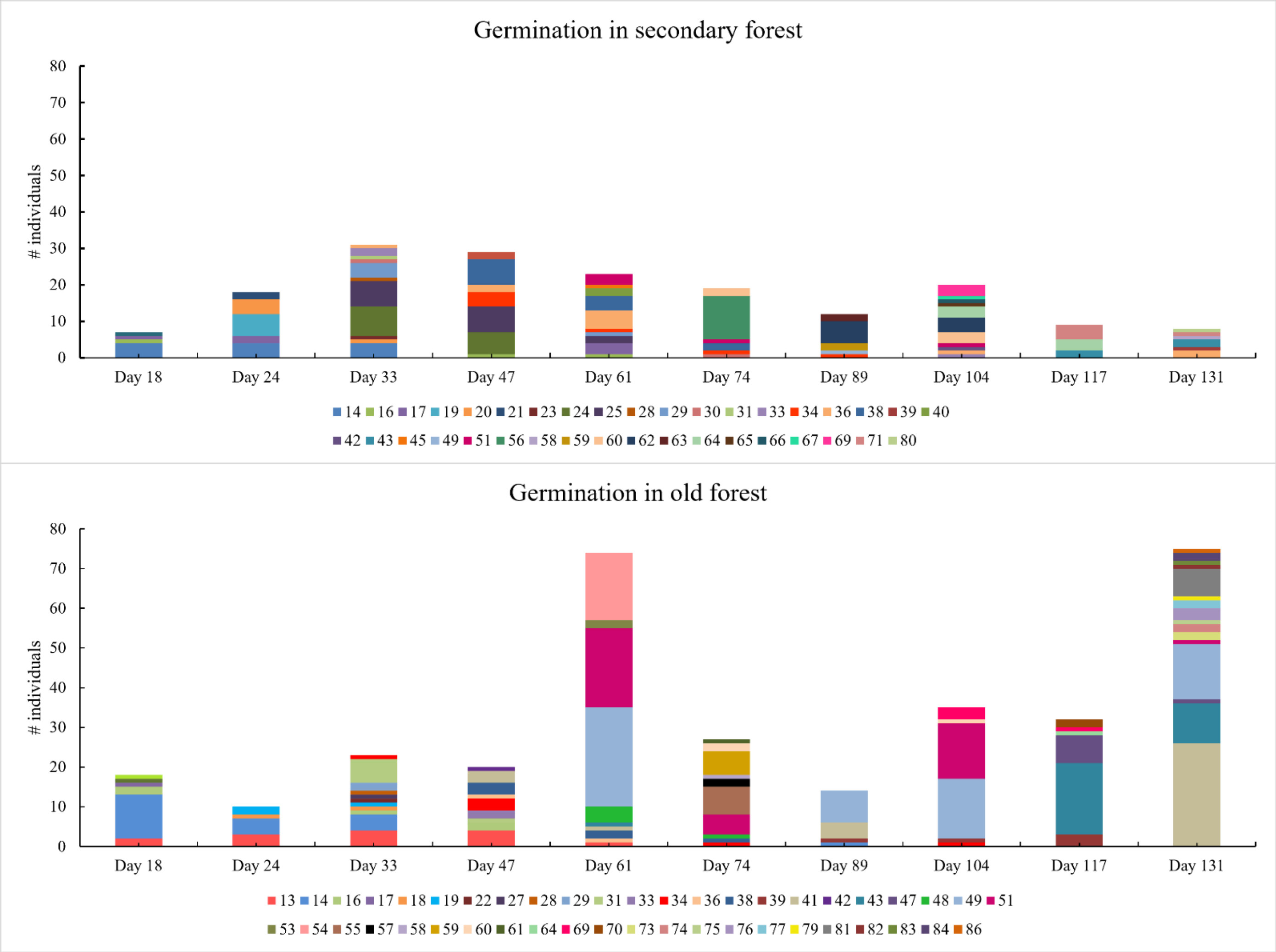

**Fig. 4.** The number of individuals germinated for the soil seed banks (SSB) of the three SF and OF sites. Each color represents a different morphotype (Table 1A Supplementary material). We excluded the morphotype number 26 (*Ulex europaeus*) (Fig. 2AA Supplementary material). Bray-Curtis Index for the SF SSB and the OF SSB = 0.48.

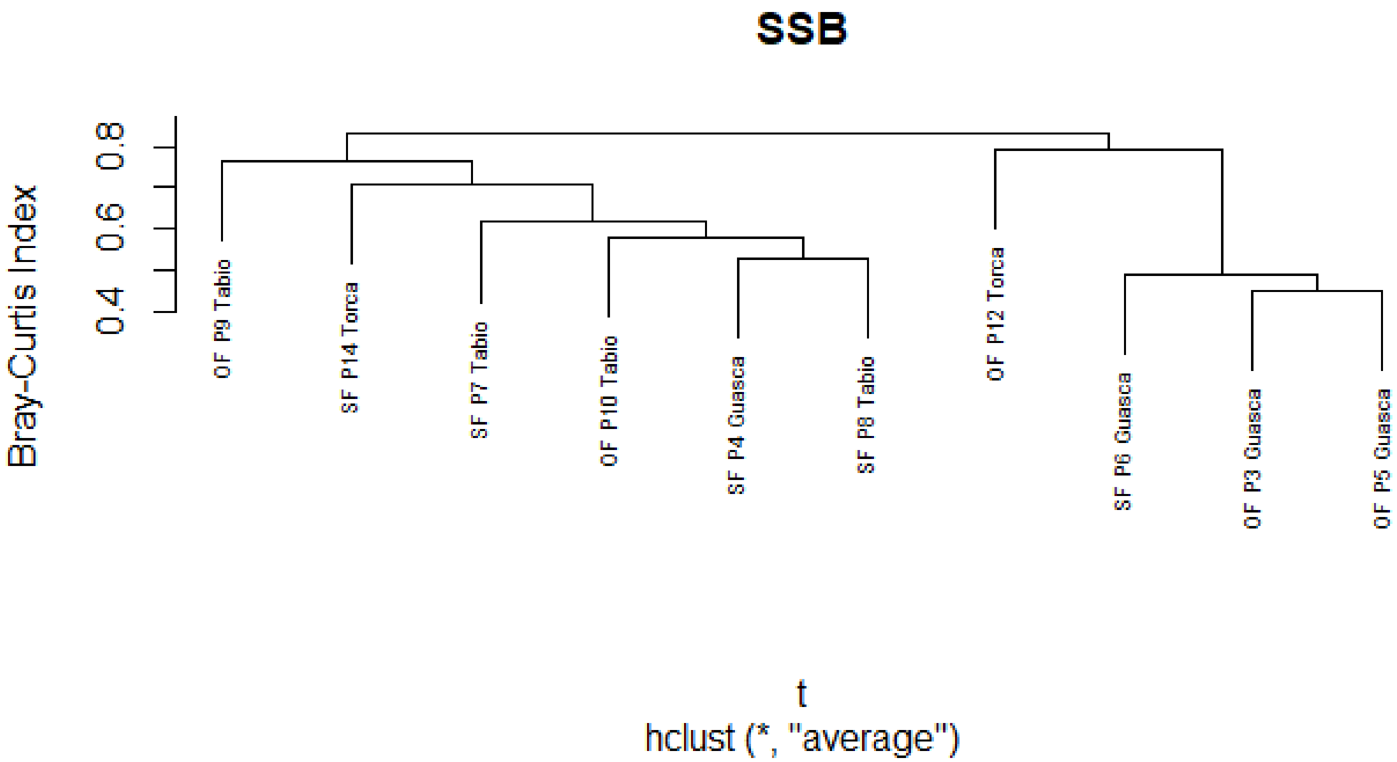

**Fig.** 5. The cluster of the soil seed bank (SSB) groups the different plots according to their similarity in species composition. We used the Bray-Curtis Index, to evaluate how much they are alike. Define SF and OF as well as “P”

There was a significant difference in community composition in relation to NH4-N flux (p-value = 0.010 – Table 4; Fig. 6). In other words, as NH4-N changed between plots, community composition also changed. Moreover, this factor (NH4-N flux) explains a significant part of the variation at the community level ($r^2$ = 0.9148). Likewise, pH was also significant ($r^2$ = 0.7607; p-value =0.027; Table 4; Fig. 6) and explained 0.76 of the variation in community composition.

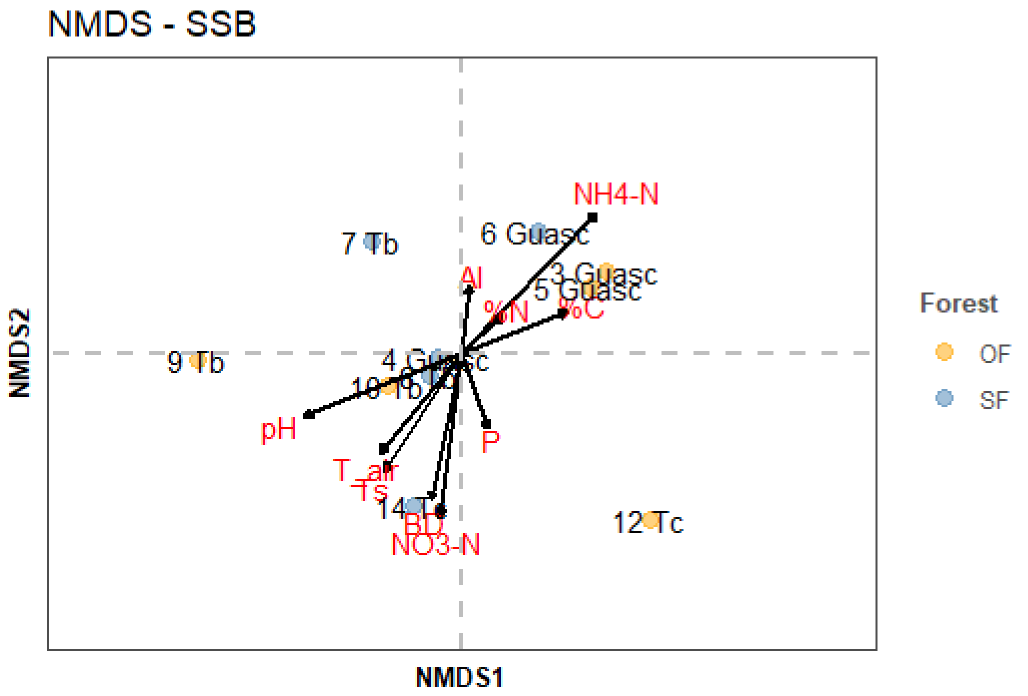

**Fig. 6** NMDS for Soil Seed Bank. The number represents the plot number, and the letters represent the site (Tb: Tabio, Guasc: Guasca, Tc: Torca). T_air: temperature at 12 cm from the soil surface, Ts: temperature at 6 cm soil depth, Al: aluminum flux (ug/10cm/30days), P: phosphorus flux, NH4-N: ammonium flux, NO3-N: nitrate flux, C%: carbon soil content, N%: nitrogen soil content; BD: bulk density (g/cm$^3$). Orange points represent old forest plots, and blue points represent secondary forest plots. The direction of the arrows shows the direction at which the environmental factor is the maximum, and the arrow length shows the degree at which the environmental factor influences the community assembly.

I performed a Mantel test to determine if soil factors and soil and air temperatures played a role in the SSB community assemblage (Table 4). For the SSB, environmental factors interacted with the community assemblage (p-value = 0.0172); nevertheless, other factors (such as study site) could also affect the assembly. I confirmed that pH and the NH4-N flux are the soil variables that better explain community's arrangement. This means that as pH or NH4-N flux differ between the plots, SSB community composition also varies (p-value = 0.0023).

**Table 4.** NMDS values for correlation of the interactions between the samples and environmental factors. Mantel (Statistic: R$^2$) and ANOSIM (Statistic: R) test to determine influence of the environmental factors

in the community assemblage. We did not perform the Mantel test for the pH and NH4-N in the seedling community because we did not obtain any significant association in the NMDS.

| | | **SSB** | | **Seedlings** | |
|---|---|---|---|---|---|
| | **Drivers of community variation** | **$R^2$ /R** | **p-value** | **$R^2$** | **p-value** |
| *NMDS* | C% soil | 0.343 | 0.501 | 0.569 | 0.167 |
| | N% soil | 0.074 | 0.905 | 0.315 | 0.506 |
| | NO3-N | 0.326 | 0.435 | 0.537 | 0.234 |
| | NH4-N | 0.915 | 0.010* | 0.725 | 0.065 |
| | P | 0.157 | 0.794 | 0.054 | 0.894 |
| | Al | 0.106 | 0.775 | 0.023 | 0.961 |
| | Bulk density (BD) | 0.477 | 0.275 | 0.613 | 0.175 |
| | pH | 0.761 | 0.027* | 0.678 | 0.087 |
| | Soil temperature (Ts) | 0.413 | 0.346 | 0.896 | 0.011* |
| | Air temperature seedlings level | 0.365 | 0.402 | 0.841 | 0.022* |
| | Type of forest | 0.092 | 0.567 | 5e-04 | 1 |
| | Site | 0.570 | 0.020* | 0.792 | 0.02* |
| *Mantel test* | All environmental factors | 0.349 | 0.017* | 0.142 | 0.162 |
| | NH4-N | 0.423 | 0.006* | - | - |
| | pH | 0.437 | 0.004* | - | - |
| | Soil temperature (Ts) | - | - | 0.602 | 0.001* |
| | Air temperature | - | - | 0.634 | 3e-04* |
| *ANOSIM test* | Sites | 0.587 | 0.002* | 0.990 | 8e-04* |

*Seedlings:*

## *A.* Seedlings' richness and diversity:

A total of 97 seedlings morphotypes were identified in the seedling survey of the 40 subplots (four per plot). 37 belonged to old forests only, 28 to the secondary forests, and 32 were shared (Table 5). The site with the highest number of seedling species was Tabio (55 morphotypes), specifically in secondary forest plots. In contrast, Torca had the lowest number of species (19 morphotypes). No morphotype was present in all six treatments (site + SF or site + OF). Nevertheless, *Rhamnus goudotiana* was present in five treatments and absented only in the subplots of the old forest in Guasca. The same happened with *Palicourea angustifolia* and *Myrsine coriaceae,* which were present in five treatments but were missing in SF Torca plots (Table 2A – Supplementary material).

**Table 5.** Number of seedling morphotypes identified in the field.

| Site | Secondary forest (SF) | Old forest (OF) | Shared morphotypes (SF – OF) | Total |
|---|---|---|---|---|
| **Seedlings (all)** | 60 | 69 | 32 | 97 |
| **Guasca** | 24 | 28 | 7 | 45 |
| **Tabio** | 40 | 36 | 21 | 55 |
| **Torca** | 7 | 15 | 3 | 19 |

In terms of richness, the community of seedlings in the old forest had a slightly greater value (18.8 ± 5.7 – Table 3) compared to secondary forests (17.6 ± 7.9; Table 3), albeit not significant (t = -0.28; p-value = 0.79; Table 3). No significant differences were found in terms of species dominance (I. Simpson Index) (t =0.49; p-value = 0.96; Table 3) or species diversity (Shannon Index) (p-value = 0.85; Table 3) between mature and secondary forest seedling communities. Finally, for the two-way ANOVA conducted for the site and type of forest, I obtained a significant difference in terms of richness (p-value = 0.0208) specifically regarding the site (Table 4A1 - Supplementary material). According to the Tukey test, Tabio (24.25 ± 2.87) and Guasca (15.75 ± 4.11) had significant differences (p-value = 0.0423466 -Table 4A 2 – Supplementary material), as well as Torca (11 ± 5.66) and Tabio (p-value = 0.0141717 - Table 4A 2 – Supplementary material).

## B. Seedling (Sdl) community composition and soil factors:

I found a stronger similarity in species composition between sites than between type of forest for the seedling community (Fig. 7). In contrast with the arrangement of the SSB, here I found that P12 Torca and P3 Guasca showed a value of 0.86 for Bray-Curtis Index, which means greater dissimilarity between these two sites and explains why these two sites are not together in seedling dendrogram (Fig. 7). Also, I obtained a 0.514 value for the Bray-Curtis Index between the community of seedlings of secondary and old forests, which means they show an intermediate dissimilarity. Additionally, in terms of composition similarity, the SSB plots arrange differently (Fig. 5) than the seedling community (Fig. 7).

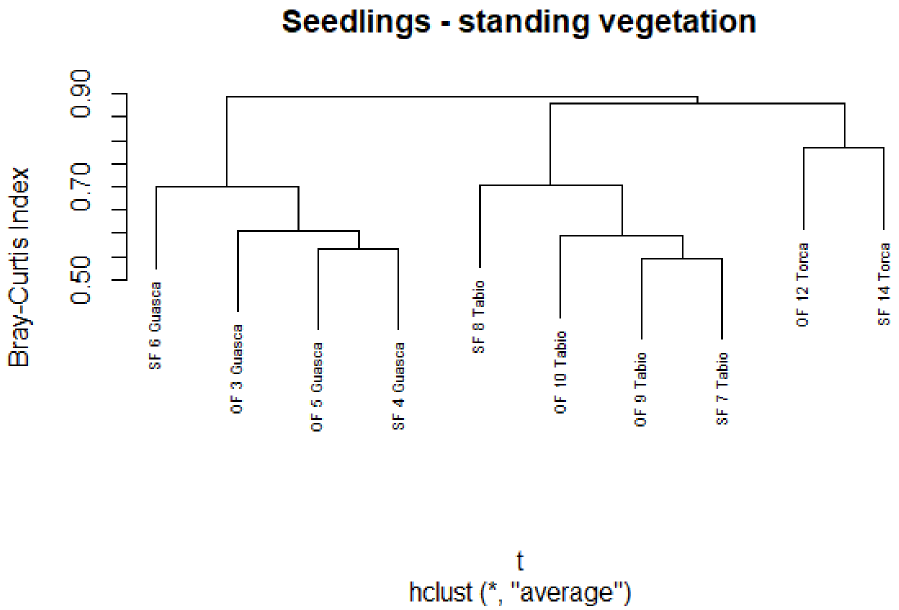

**Fig. 7.** Cluster of the seedling community groups from different plots according to their similarity in species composition, according to the Bray-Curtis Index. Bray – Curtis between OF-Sdl and SF-Sdl= 0.51. Define what the abbreviations are.

To test if some soil factors could play a role in the establishment of the community of seedlings, I performed an NMDS combined with a Mantel Test. I found no significant differences in the community structure regarding any of the soil components analyzed (p-value (Mantel Test) = 0.1622; Table 4). Nevertheless, I found that soil and air temperatures were significantly different (p-value = 0.001 and 3e-04, respectively; Table 4).

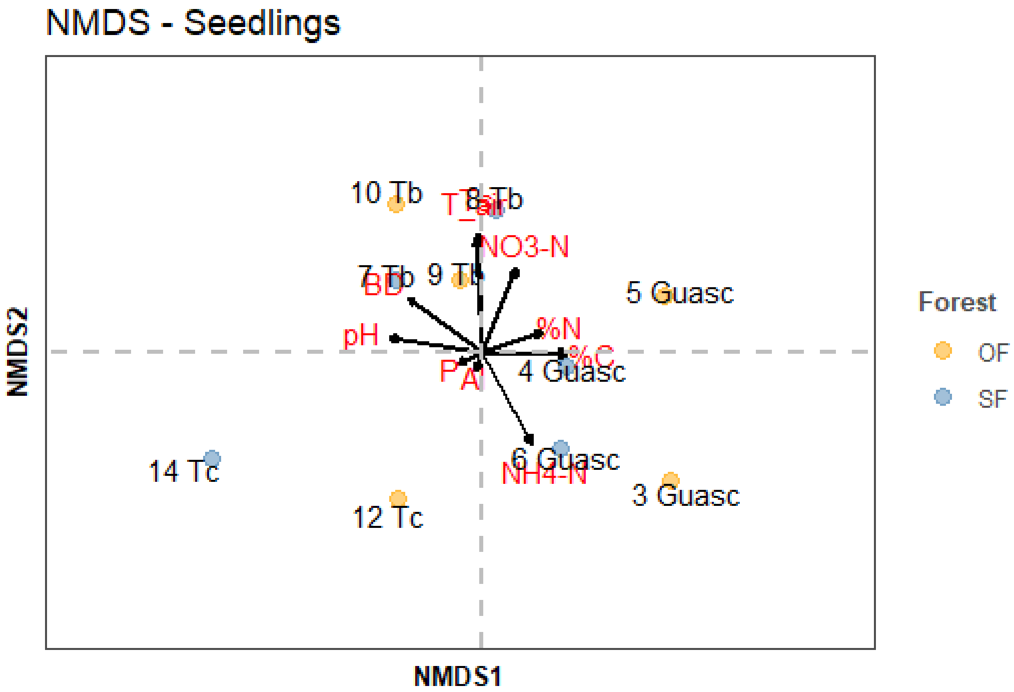

**Fig. 8.** NMDS for the community of seedlings. Plots are shown: the number represents the plot number, and the letters represent the site (Tb: Tabio, Guasc: Guasca, Tc: Torca). Orange points represent old forest plots, and blue points represent secondary forest plots. T_air: temperature at 12cm from soil surface and Ts: temperature at 6cm soil depth

Some factors are more associate with the seedling community assemblages of the different plots. For example, NH4-N ($R^2$ = 0.7246 - Table 4) soil flux represents a greater association than P ($R^2$= 0.0538 – Table 4) or Al ($R^2$ = 0.0234 – Table 4) content (Fig. 8). Finally, site played the most important role in the community assemblage (p-value = 8e-04).

## 5. Discussion:

### *Soil Seed Bank:*

I hypothesized that there would be similarities in the community composition of the soil seed bank (SSB) between SF and OF, since SSB is mainly composed of persistent seeds which are primarily from pioneers' (Dalling & Brown, 2009). Yet, the Bray-Curtis index between these two groups was 0.48, which means an intermediate similarity. Even though SF and OF SSB have a total of 40 different species, they share 22 of them, partially supporting our hypothesis.. This is partially consistent with Daïnou et al., (2011) who found that SSB is mainly composed of pioneers species and woody species from mature forests are not stocked in the soil seed bank. In other words, SF and OF SSBs are not so different in community composition. In ours results, morphotype number 43, from the genus *Myrsine*, had a peak of germination during days 117 and 131 (Fig. 4) and was found mostly in old forests. This genus includes some trees, but mostly shrubs, who are commonly present in secondary forest (Siminski et al., 2011), meaning that the SSB of mature forest is also composed of species characteristic of secondary forests.

Nevertheless, since I obtained an intermediate similarity between SF and OF SSBs (Bray-Curtis = 0.48), I can also affirm that there are some differences in the species community composition of the forests. In the germination process, several morphotypes in the OF had a late germination peak (Fig. 4). As there is a late germination peak only present in mature forests (day 131), an alternative interpretation is that the SSB in mature Andean Forests include dormant species and late successional species could be present as well. Besides, at OF plots I found morphotypes that were exclusive to that type of forest (Fig. 4), such as numbers 54 and 81 (Fig. 2A – Supplementary material). As I was not able to identify all species, it is not possible to tell which morphotypes correspond to late-successional species.  Nonetheless, Gelviz-Gelvez et al., (2016) found in their SSB analysis in Northern Colombia that some species such as *Gaiadendron punctatum* and *Smilax sp*. were only present in the SSB of old forest, meaning that late successional species are mostly found at old forest SSB, even though late successional species appear to be scarce. Also, in terms of richness and diversity index (Shannon-Weiner and Inverse Simpson), I did not find any significant differences. Nonetheless, as I calculated these indexes for each plot, I only had an n = 5 for each group (five plots of SF and five plots of OF), so it would be interesting to replicate the analysis including a larger number of plots.

I found that site played a more important role in the SSB community assemblage than the type of forest (Fig. 5, 6). These results can be related to the current dispersal of propagules from the local community and more distant sources since the assemblage of the SSB community is strongly influenced by dispersion dynamics (Daïnou et al., 2011). At each site, dispersals can vary and change the SSBs. For example, Torca is close to some suburbs of Bogotá and near some motorways (Calbi et al., 2020). Because of that environment, many animal dispersers that are essential dispersal vectors for large seeds may avoid the zone (Lippok et al., 2013), causing low diversity.

On the other hand, Tabio is part of the "Reserva Forestal Protectora Productora de la Cuenca Alta del Río Bogotá" where restoration increases the connectivity of different forest patches (Calbi et al., 2020). That facilitates the mobility of different dispersers and the arrival of new species. Also, due to this new connectivity between forest fragments, it is possible that the SSB composition does not vary significantly between plots (Fig. 5). On the other hand, the disturbance previous to the secondary forest's appearance can modify the SSB's composition (Lippok et al., 2013). Guasca had limestone mining activity until the late 1990s (Hurtado-M et al., 2021), and Machado et al., (2021) found that species richness and diversity tend to be lower in mining areas; besides they found three species that were only in the SSB of the mining area. Two of them were phyto-remediating species (*Gomphrena claussenii* and *Leucaena leucocephala*) and the third one was an invasive species (*Calotropis procera*) which is characterized by being resistant to different environmental conditions (Machado et al., 2021). This is consistent with our results for Guasca, where there is a high abundance of *Ulex europaeus* possibly due to their capacity of adapting to multiple environmental conditions.

In addition, Torca showed the lowest values in seed density. Most of the species that are currently establishing in Torca could be late-successional that are characterized by having transient seeds which do not contribute to the SSBs (Daïnou et al., 2011). Additionally, at Torca plots leaf litter was around 30 cm. This could also affect the seed input since it acts as a barrier and causes a reduction in seed density (Lippok et al., 2013).

Apart from the site, pH also played a role in the community assemblage of the soil seed bank (Table 4). pH can be related to the persistence and viability of the seeds. At acidic pHs only a few seed species can avoid or resist physical damage (Pakeman et al., 2012). In contrast, other studies have shown that seed persistence decreases with increasing pH due to an increase in the presence of microorganisms that affect the viability of seeds (Basto et al., 2015). However, both studies propose that seed survival regarding pH is species specific (Basto et al., 2015; Pakeman et al., 2012), implying that differences between the plots in soil pH leads to variation in the seed community.

Finally, NH4-N was also important in the community assemblage of the SSB (Table 4). NH4-N is important in the germination process (Zhong et al., 2019) since each species respond differently to the addition of N (Ochoa-Hueso & Manrique, 2010). Zhong et al., (2019) found that with the addition of N the density and richness of some dominant species decrease. Besides, nitrogen availability tends to promote pioneer species germination (Luna & Moreno, 2008). Therefore, variation between plots in terms of nitrogen fluxes, may promote differences in species germination.

Seedlings:

I hypothesized that there would be differences in the seedling community composition in the two types of forest, since in old forests shade-tolerant species can successfully establish due to the canopy closure, while in young forests, pioneers are more frequent (Chazdon, 2017). Nonetheless, the Bray-Curtis Index showed an intermediate dissimilarity between both ecosystems (Fig. 7). Thus, OF and SF share many species. Also, as I could not identify all the morphotypes, it was difficult to determine at this stage which are pioneer's species, and which are late successional ones. On the other hand, only two species were shared between the three sites of study: *Palicourea angustifolia* and *Myrsine coriaceae* (Table 2A – Supplementary material). The first one is characterized by being a fast-growing species with high dispersal capacity (García & Torres-González, 2021), characteristic of a pioneer species. The second is used in silvicultural restoration, since its establishment and reproduction are very easy (Cantillo Higuera et al., 2008).

Forest type was not a determining factor in the assembly of seedling communities (Table 4). Nonetheless, the site showed an important role in the community assemblage (Table 4). This is consistent with what Hurtado-M et al. (2021) reported, as they found that in secondary and old forests near Bogota, site was the main driver of floristic composition while forest successional status has a smaller effect on seedling community composition. Also, they only found three seedling species that were shared between the six study sites, which is consistent with what we found here, where only three species occurred across the three sites (i.e., *Rhamnus goudotiana*, *Palicourea angustifolia,* and *Myrsine coriaceae*). As Hurtado-M et al. (2021) proposed, the species composition is "idiosyncratic". In other words, arrangement of species does not follow a general pattern and it shifts according to sites and among plots.

Furthermore, seedling compositional variation was not sensitive to changes in soil nutrients (Table 4). That is not consistent with what Hurtado-M et al., (2021) found, where P appeared to be a critical driver of seedling compositional variation. Also, some studies expose that in terms of seedlings establishment, nutrients are less critical than limited light availability (Holste et al., 2011). Thus, it would be interesting to determine the role of light availability and canopy opening in community assemblage.

Finally, I found that soil temperature played a role in the variation of seedling community composition (Table 4). That can be related to the emergence capacity of each species (Dubetz et al., 2011). At higher soil temperature, seedling emergence tends to increase (Awal & Ikeda, 2002; Dubetz et al., 2011). Nevertheless, that depends on the species identity, since some of them tend to emerge at lower temperatures (Singh & Dhaliwal, 1972). Some studies have proposed that at higher temperatures, species richness tend to decrease even though the number of emerging seedlings stayed the same (Lloret et al., 2004). As shown in this study, the differences between plots in terms of soil temperature, translate into variation in the seedling community.

### *Seed Soil Bank vs. seedlings:*

For the SSB assessment and the seedlings evaluation (Fig. 5 and Fig. 7) plots do not arrange in the same way (Fig. 5 and Fig. 7) regarding similarity. Consequently, I hypothesize that the soil features and the driving forces contributing to the maintenance of a soil seed bank are not the same as the factors needed in seedlings establishment. This is consistent with the results from Gomes, Oliveira, Rocha Miranda, et al. (2019), which found that of 166 species found in the standing vegetation of a dry forest in Brazil, only 50 species occurred in the SSB. Therefore, we could say that SSB is made up of seeds characterized by their capacity to persist in the soil (Daïnou et al., 2011) and that can be affected by soil pH and NH4-N soil flux. As Daïnou et al. (2011) reported, the SSB community was mostly composed by pioneers and herbaceous species with few shade-tolerant species. In contrast, the community of seedlings can be more influenced by the dispersal activity, the seed rain (Gomes et al., 2019), shade-tolerance and soil temperature.

Finally, as my study focused on finding natural patterns in community assemblage, I did not consider the germination of *Ulex europaeus.* Nonetheless, I observed a germination peak of 152 individuals of this specie on day 33 and 47 (Table 2A – Supplementary material). Meanwhile, in the seedling community, I only identify four individuals, which means that *U. europaeus* is not currently establishing in our plots, even if it is a representative specie in the SSB. Therefore, further studies need to be done to evaluate the role of this invasive species in regenerating forests and how it can be included in better management programs.

Considerations:

Additional research needs to be done on the composition of seed communities in the litter because that would provide information regarding dispersion rates that can be compared with the SSB data. Also, it is essential to mention that germination must done for longer periods of time. Finally, it would be interesting to perform this project with a more significant number of plots in each site to evidence differences between the two types of forest.

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## 12. Supplementary Material

**Table 1A**. Morphotypes identified at the SSB (Soil seed bank) of the three sampling sites. OF: old forest and SF: secondary forest. We only identify six morphotypes (13, 14, 26, 42. 54, 43)

| | **Guasca** | | **Tabio** | | **Torca** | |
|---|---|---|---|---|---|---|
| **Morphotypes** | **OF** | **SF** | **OF** | **SF** | **OF** | **SF** |
| 16 | | 1 | 3 | 2 | 2 | |
| 14 *(Bocconia frutescens)* | | | 20 | 12 | | |
| 17 | | | 1 | 5 | | 1 |
| 13 *(Croton bogotanus*) | | | 14 | | | |
| 20 | | 4 | | | | 1 |
| 21 | | 2 | | | | |
| 19 | 1 | 4 | 2 | 2 | | |
| 18 | 2 | | | | | |
| 26 (*Ulex europaeus*) | 2 | 307 | | 7 | | |
| 25 | | 16 | | | | |
| 23 | | 1 | | | | |
| 24 | | 14 | | | | |
| 22 | 1 | | | | | |
| 27 | 1 | | | | | |
| 28 | 1 | | | 1 | | |
| 31 | | | 7 | 1 | | |
| 36 | | 2 | 2 | 8 | | 1 |
| 29 | | 1 | 2 | 3 | | 1 |
| 33 | | 1 | 2 | 2 | | |
| 30 | | | | 2 | | |
| 34 | 4 | 4 | 2 | 3 | | |
| 38 | 4 | 3 | 2 | 10 | | |
| 39 | 1 | 2 | 4 | | | 1 |
| 41 | 1 | | 33 | | | |
| 42 (*Rhamnus goudotiana*) | | | 1 | | | 1 |
| 53 | 2 | | | | | |
| 51 | 39 | 4 | 1 | 1 | | |
| 45 | | | | 1 | | |
| 54 Cf *Rhamnus* | | | 17 | | | |
| 48 | | | 5 | | | |
| 49 | | | 62 | 1 | | |
| 43 (*Mysine*) | 17 | 1 | 1 | 2 | 11 | 1 |
| 40 | | | | | | 2 |
| 61 | | | 1 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 60 | | | 3 | 5 | | |
| 57 | | | 2 | | | |
| 55 | 6 | | | | 1 | |
| 59 | 6 | 2 | | | | |
| 58 | 1 | 1 | | | | |
| 56 | | | | 12 | | |
| 62 | | 10 | | | | |
| 63 | | | | 2 | | |
| 64 | | | 1 | 6 | | |
| 65 | | | | 1 | | |
| **66** | | | | 1 | | |
| **67** | | | | 1 | | |
| **69** | 4 | 3 | | | | |
| **70** | 2 | | | | | |
| **71** | | 4 | | 1 | | |
| **47** | | | 8 | | | |
| **73** | 2 | | | | | |
| **74** | 2 | | | | | |
| **75** | 1 | | | | | |
| **76** | | | 3 | | | |
| **77** | | | 2 | | | |
| **79** | | | 1 | | | |
| **81** | | | 7 | | | |
| **82** | | | 1 | | | |
| **83** | | | 1 | | | |
| **84** | | | 2 | | | |
| **86** | | | 1 | | | |
| **80** | | | | 1 | | |

**Table 2A**. Morphotypes identified in the seedling survey of the three sampling sites. OF: old forest and SF: secondary forest. We label the morphotypes by putting them a number, nevertheless we were able to identify some of the species..

| | **Guasca** | | **Tabio** | | **Torca** | |
|---|---|---|---|---|---|---|
| **Morphotypes** | **OF** | **SF** | **OF** | **SF** | **OF** | **SF** |
| 34 | 6 | | 1 | | | |
| 35 | 2 | | 1 | | | |
| 43 | 1 | 1 | | | | |
| 44 | 5 | | | | | |
| 45 | 1 | | | | | |
| 46 | 1 | | | | | |
| 48 | 2 | | | | | |
| 49 | 1 | | | | | |
| 50 | 1 | | | | | |
| 51 | 2 | | | | | |
| 52 | 1 | | | | | |
| 53 | 1 | | | | | |
| 54 | 2 | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Cestrum buxifolium* | 1 | | | | | |
| *Cybianthus iteoides* | 3 | 1 | | | | |
| *Diplostephium rosmarinifolius* | 1 | | | | | |
| *Hesperomeles goudotiana* | 2 | | | 1 | | |
| *Macrocarpaea glabra* | 1 | | | | | |
| *Miconia ligustrina* | 12 | 9 | 3 | 1 | | |
| *Myrsine latifolia* | 1 | | | 2 | | |
| *Myrsine coriaceae* | 37 | 47 | 2 | 2 | 2 | |
| *Myrsine dependens* | 1 | | | | | |
| *Oreopanax bogotensis* | 1 | | | | | |
| *Palicourea angustifolia* | 25 | 2 | 3 | 14 | 4 | |
| *Piper artanthe* | 4 | 4 | | | | |
| *Piper bogotense* | 1 | | 8 | 17 | | |
| *Symplocos theoformis* | 1 | | | | | |
| *Weinmannia tomentosa* | 6 | 10 | | | | |
| 19 | | | 11 | 1 | | |
| 26 | | | 1 | 2 | | |
| 29 | | | 8 | | | |
| 30 | | | 1 | | | |
| 31 | | | 3 | | | |
| 33 | | | 1 | | | |
| 36 | | | 1 | | | |
| 37 | | 2 | 1 | | | |
| 38 | | | 1 | | | |
| 39 | | | 1 | | | |
| 40 | | | 1 | | | |
| 41 | | | 1 | | | |
| 42 | | | 1 | | | |
| 60I | | | 2 | | | |
| *Cedrela montana* | | | 88 | 2 | | |
| *Croton bogotanus* | | | 1 | | | |
| *Daphnopsis caracasana* | | | 32 | 57 | | 1 |
| *Duranta mutissi* | | | 1 | 20 | | |
| *Miconia squamulosa* | | | 4 | 70 | | |
| *Myrcianthes leucoxyla* | | | 1 | 6 | | |
| *Myrcianthes rhopaloides* | | | 1 | 2 | | |
| *Oligactis sessiliflora* | | | 1 | 1 | | |
| *Oreopanax incisus* | | 2 | 12 | 5 | | |
| *Palicourea lineariflora* | | | 2 | 3 | | |
| *Psychotria boqueronensis* | | | 3 | 24 | | |
| *Rhamnus goudotiana* | | 2 | 11 | 17 | 4 | 17 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Vallea  stipularis* | | 3 | 1 | 1 | | |
| *Varronia cylindrostachya* | | | 4 | 7 | | |
| *Viburnum triphyllum* | | 11 | 19 | 32 | 4 | |
| *Xylosma spiculifera* | | 4 | 22 | 22 | | |
| 13 | | | | 3 | 3 | |
| 58 | | 1 | | | 1 | |
| 61 | | | | | 10 | |
| 62 | | | | | 1 | |
| 63 | | | | | 1 | |
| *Ageratina asclepiadea* | | | | | 1 | 6 |
| *Aiouea dubia* | | | | | 1 | |
| *Cavendishia bracteata* | | | | | 1 | |
| *Clusia multiflora* | | | | | 1 | |
| *Drimys granadensis* | | | | | 2 | |
| *Prunus buxifolia* | | | | | 3 | 3 |
| 47 | | 2 | | | | |
| 55 | | 1 | | | | |
| 56 | | 1 | | | | |
| 57 | | 1 | | | | |
| 59 | | 1 | | | | |
| 60 | | 2 | | | | |
| 7 | | 4 | | 1 | | |
| *Ageratina glyptophlebia* | | 1 | | | | |
| *Gaiadendron punctatum* | | 3 | | | | |
| *Ulex europaeus* | | 4 | | | | |
| 1 | | | | 2 | | |
| 10 | | | | 2 | | |
| 11 | | | | 1 | | |
| 12 | | | | 2 | | |
| 15 | | | | 1 | | |
| 16 | | | | 2 | | |
| 17 | | | | 1 | | |
| 18 | | | | 1 | | |
| 20 | | | | 2 | | |
| 21 | | | | 2 | | |
| 25 | | | | 1 | | |
| 27 | | | | 1 | | |
| 3 | | | | 4 | | |
| 4 | | | | 1 | | |
| *Matelea mutisiana* | | | | 1 | | |
| *Cletra fimbriata* | | | | | | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Gaultheria anastomosans* | | | | | | 3 |
| *Ocotea calophylla* | | | | | | 1 |

**Table 3A.** Dunn Test for plots showing significant differences in terms of seed density. Those in bold letter are plots from the same sites. P (number): plot number. OF: old forest. SF: secondary forest.

| **Plots** | **p-value** |
|---|---|
| **OF P3 Guasca – SF P6 Guasca** | 0.0049 |
| OF P10 Tabio – SF P14 Torca | 0.047 |
| SF P7 Tabio – SF P6 Guasca | 0.026 |
| OF P12 Torca – SF P4 Guasca | 0.0094 |
| **OF P3 Guasca – SF P4 Guasca** | 0.046 |
| SF P14 Torca –SF P4 Guasca | 0.002 |
| OF P12 Torca – SF P6 Guasca | 0.001 |
| SF P14 Torca – SF P6 Guasca | 0.0001 |
| **Sites** | |
| Guasca - Tabio | 1.0 |
| Tabio - Torca | 3.56e-04* |
| Guasca-Torca | 1.43e-05* |

**Table 4A - 1** Two-way ANOVA comparing Hill numbers for each sampling site (Tabio, Torca and Guasca) and forest type (Secondary and old forest).

| | **SSB** | | | | | **Seedlings** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Shapiro – wilk test** | **Site** | | **Forest** | | **Shapiro – wilk test** | **Site** | | **Forest** | |
| | | **F value** | **P-value** | **F value** | **P-value** | | **F value** | **P-value** | **F value** | **P-value** |
| **Richness (q=0)** | 0.679 | 3.409 | 0.103 | 0.048 | 0.833 | 0.692 | 7.914 | 0.0208* | 0.208 | 0.664 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Shannon index (q=1)** | 0.3075 | 1.885 | 0.2317 | 0.045 | 0.839 | 0.212 | 0.782 | 0.499 | 0.0391 | 0.8499 |
| **Inverse Simpson index (q=2)** | 0.058 | 1.137 | 0.3813 | 0.252 | 0.633 | 0.202 | 0.0623 | 0.9402 | 0.0019 | 0.9662 |

**Table 4A - 2** Tukey test comparing richness in the community of seedlings according to the site (Tabio, Torca and Guasca)

| **Sites** | p-value |
|---|---|
| Tabio – Guasca | 0.042* |
| Torca - Guasca | 0.391 |
| Torca - Tabio | 0.0141717* |

**Table 5A.** Bray-Curtis index for SSB. OF: old forest, SF: Secondary forest, P (number): plot number.

| | **OF P10 Tabio** | **OF P12 Torca** | **OF P3 Guasca** | **OF P5 Guasca** | **OF P9 Tabio** | **SF P14 Torca** | **SF P4 Guasca** | **SF P6 Guasca** | **SF P7 Tabio** | **SF P8 Tabio** |
|---|---|---|---|---|---|---|---|---|---|---|
| **OF P10 Tabio** | 0,00 | 0,86 | 0,84 | 0,77 | 0,60 | 0,65 | 0,61 | 0,78 | 0,62 | 0,55 |
| **OF P12 Torca** | 0,86 | 0,00 | 0,71 | 0,81 | 1,00 | 0,82 | 0,89 | 0,86 | 1,00 | 0,85 |
| **OF P3 Guasca** | 0,84 | 0,71 | 0,00 | 0,45 | 1,00 | 0,89 | 0,77 | 0,45 | 0,82 | 0,76 |
| **OF P5 Guasca** | 0,77 | 0,81 | 0,45 | 0,00 | 0,94 | 0,85 | 0,70 | 0,52 | 0,86 | 0,71 |
| **OF P9 Tabio** | 0,60 | 1,00 | 1,00 | 0,94 | 0,00 | 0,91 | 0,86 | 1,00 | 0,68 | 0,73 |
| **SF P14 Torca** | 0,65 | 0,82 | 0,89 | 0,85 | 0,91 | 0,00 | 0,65 | 0,89 | 0,79 | 0,74 |
| **SF P4 Guasca** | 0,61 | 0,89 | 0,77 | 0,70 | 0,86 | 0,65 | 0,00 | 0,62 | 0,69 | 0,53 |
| **SF P6 Guasca** | 0,78 | 0,86 | 0,45 | 0,52 | 1,00 | 0,89 | 0,62 | 0,00 | 0,64 | 0,71 |
| **SF P7 Tabio** | 0,62 | 1,00 | 0,82 | 0,86 | 0,68 | 0,79 | 0,69 | 0,64 | 0,00 | 0,53 |
| **SF P8 Tabio** | 0,55 | 0,85 | 0,76 | 0,71 | 0,73 | 0,74 | 0,53 | 0,71 | 0,53 | 0,00 |

**Table 6A.** Bray – Curtis index for seedlings. OF: old forest, SF: secondary forest.

| | **OF 10 Tabio** | **OF 12 Torca** | **OF 3 Guasca** | **OF 5 Guasca** | **OF 9 Tabio** | **SF 14 Torca** | **SF 4 Guasca** | **SF 6 Guasca** | **SF 7 Tabio** | **SF 8 Tabio** |
|---|---|---|---|---|---|---|---|---|---|---|
| **OF 10 Tabio** | 0 | 0,938 | 0,967 | 0,966 | 0,561 | 0,967 | 0,885 | 0,951 | 0,633 | 0,776 |
| **OF 12 Torca** | 0,938 | 0 | 0,863 | 0,906 | 0,893 | 0,778 | 0,855 | 0,781 | 0,845 | 0,914 |
| **OF 3 Guasca** | 0,967 | 0,863 | 0 | 0,626 | 0,94 | 1 | 0,63 | 0,706 | 0,96 | 0,941 |
| **OF 5 Guasca** | 0,966 | 0,906 | 0,626 | 0 | 0,929 | 1 | 0,575 | 0,805 | 0,953 | 0,892 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **OF 9 Tabio** | 0,561 | 0,893 | 0,94 | 0,929 | 0 | 0,889 | 0,865 | 0,85 | 0,538 | 0,75 |
| **SF 14 Torca** | 0,967 | 0,778 | 1 | 1 | 0,889 | 0 | 0,983 | 0,97 | 0,82 | 0,99 |
| **SF 4 Guasca** | 0,885 | 0,855 | 0,63 | 0,575 | 0,865 | 0,983 | 0 | 0,697 | 0,897 | 0,914 |
| **SF 6 Guasca** | 0,951 | 0,781 | 0,706 | 0,805 | 0,85 | 0,97 | 0,697 | 0 | 0,891 | 0,892 |
| **SF 7 Tabio** | 0,633 | 0,845 | 0,96 | 0,953 | 0,538 | 0,82 | 0,897 | 0,891 | 0 | 0,638 |
| **SF 8 Tabio** | 0,776 | 0,914 | 0,941 | 0,892 | 0,75 | 0,99 | 0,914 | 0,892 | 0,638 | 0 |
| | | | | | | | | | | |

**Table 7A.** Normality test for seed density in the different study groups.

| | **Kolmogorov-Smirnov Test (p-values)** | **Levene Test** |
|---|---|---|
| **Old forest** | 1.107e-10 | 0.2535 |
| **Secondary forest** | 1.193e-08 | |
| **Tabio** | 3.343e-06 | 0.01186 |
| **Guasca** | 5.104e-07 | |
| **Torca** | 1.23e-06 | |

**Fig. 2A** Pictures of morphotype number 54 (B) and 81 (A) germinated to evaluate the SSB (soil seed bank).

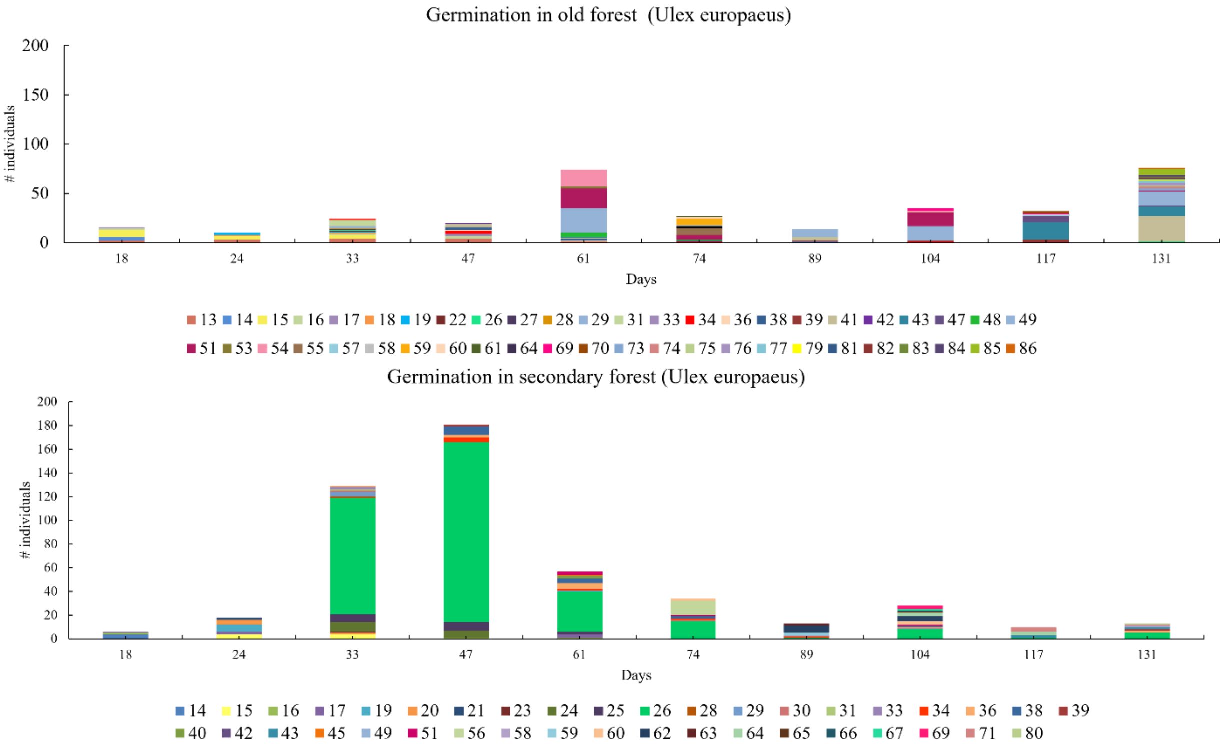

**Fig. 2AA** The number of individuals germinated for the soil seed banks (SSBs) of the three secondary and old forest sites. Each color represents a different morphotype. We included the morphotype number 26 (*Ulex europaeus*). Bray-Curtis Index for the SF SSB and the OF SSB = 0.4761905.