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Modification of Density Dependence and Habitat Filtering on Seedling Survival of Different Mycorrhizal-Type Tree Species in Temperate Forests

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Abstract: Conspecific negative density dependence (CNDD) and habitat filtering are critical to seedling survival. However, the relative importance of the two processes in affecting survival of seedlings with different types of mycorrhizae remains unclear. In this study, the effects of CNDD and habitat filtering on the survival of tree seedlings with different mycorrhizal types were investigated at different successional stages of a temperate forest in the Changbai Mountain Natural Reserve, Northeast China. Conspecific negative density dependence and habitat filtering significantly affected seedling survival. In the early successional stage, the interactions between conspecific neighbor tree density and light availability and soil properties significantly negatively affected survival of all species and arbuscular mycorrhizal (AM) seedlings in the community, but not that of ectomycorrhizal (EcM) seedlings, and the CNDD effect was stronger on AM seedlings than on EcM seedlings. In the mid-successional stage, CNDD effects were stronger on EcM seedlings. Therefore, different types of mycorrhizal seedlings responded differently to CNDD and habitat filtering mechanisms during community succession, and thus, tree mycorrhizal association could determine the effects of CNDD and habitat filtering on seedling survival in temperate forests.

Keywords: tree seedling survival; conspecific negative density dependence; habitat filtering; mycorrhizal types; arbuscular mycorrhizae; ectomycorrhizae; temperate forest succession; light availability; soil properties

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1. Introduction

Mechanisms of community assembly and biodiversity maintenance are core areas of research in ecology. Habitat filtering is the overall process of selecting which individuals will contribute to future communities, and conspecific negative density dependence (CNDD) can be the most important process involved in habitat filtering. Specifically, CNDD predicts that adult neighbors will inhibit the survival of conspecific offspring because of intraspecific competition or shared host-specific enemies [1–4], which ultimately benefits the survival of hetero-species and increases local diversity. Although numerous studies confirm the importance of density-dependent effects [5–8], a consensus has not been reached because of high variability in local habitat conditions, forest types, and species strategies. Therefore, a comprehensive study is needed to reveal how the effects of CNDD vary over local environmental gradients where different species co-occur.

The effects of CNDD on seedling survival are partly determined by mycorrhizal fungal activity, which can increase nutrient absorption and provide protection for host plants [9]. The two main types of mycorrhizae, arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) fungi, should influence CNDD strength among host plant species [10]. Ectomycorrhizal seedlings perform better (i.e., show positive plant–soil feedback) when grown in soil near (or regulated by) the same species, whereas AM seedlings have the opposite response.

Compared with AM forests, the positive feedback mechanism proposed in ECM forests can better protect seedlings of the same species from soilborne pathogens and improve nutrient access [11]. However, in a study of 15 temperate forest communities in the US and China, Jiang et al. [12] found that CNDD effects due to AM species were stronger than those due to EcM species in most forests. In North American temperate forests, Bennett et al. [10] reached a similar conclusion, which may be because EcM fungi can uptake more nitrogen from the environment and transfer it to the host than AM fungi. Simultaneously, EcM fungi form an outer physical sheath, which provides greater protection from antagonists than that of AM fungi. However, in temperate forests, species typically have different growth patterns. In some studies [13,14], CNDD effects were stronger for seedlings or saplings of EcM species than for those of AM species in temperate forests. Therefore, additional studies are needed to explore the effects of CNDD on survival of seedlings with different mycorrhizal associations in temperate forests.

Effects of CNDD on seedling survival are also restricted by environmental conditions. Light and soil properties are important abiotic factors that directly affect seedling dynamics [3]. When soil and light resources are scarce, competition for resources between plants increases in intensity [15], pathogen virulence increases, and plant defensive ability decreases [16]. Therefore, CNDD effects are strongest in areas with low light and poor soil. Holík et al. [16] found that seedling mortality increased with increases in neighboring seedling species under low light levels. However, LaManna et al. [17] found that the intensity of negative density effects increases with the availability of resources at both seedling and sapling stages in a temperate forest. In other regional-scale studies, CNDD intensity in woody plant species increases with precipitation and productivity [2,18]. In addition, Johnson et al. [18] found that CNDD intensifies in low-latitude temperate forests in the eastern US. Therefore, it remains unclear how the effects of CNDD vary with local environmental conditions.

Forest successional stage must also be considered to understand the effects of CNDD on seedling survival [19,20] because of important differences in forest structure, soil conditions, and light availability [21]. For example, light has significant effects on seedling survival in early stages of succession with open canopies, but its influence diminishes with canopy closure in late stages [22]. In addition, stand density and species aggregation may decrease with succession, which weakens density-dependent effects on seedling survival [23]. Jiang et al. [24] showed that fungi are important regulators of nutrient cycling processes during succession in temperate forests and that the relative abundance of EcM fungi increases significantly with succession. Therefore, EcM species may have insignificant or positive density-dependent effects in late-succession forests. However, how species with different mycorrhizal associations respond to CNDD along a successional gradient remains unclear.

The aims of this paper were (1) to investigate the relative importance of density dependence and environmental factors on seedling survival in different successional stages of a temperate forest and (2) to compare the responses of species with different mycorrhizal types to those drivers. This study addresses two specific questions: (1) how do CNDD, soil nutrients, and light availability influence seedling survival in different successional stages? (2) Do effects of CNDD, soil nutrients, and light availability depend on mycorrhizal type?

2. Methods

2.1. Study Site and Data Collection

Three forest plots were established in the Changbai Mountain Natural Reserve in Jilin Province, Northeast China. The region has a northern temperate continental monsoon climate, with long, cold winters and short, cool summers. Average annual temperature ranges from 2 °C to 5 °C, and annual cumulative precipitation ranges from 600 to 1000 mm [25].

Within the reserve, vegetation types and diversity vary significantly with elevation. In low-elevation areas (500–1100 m), temperate mixed coniferous forests are the dominant vegetation type, and the climax community is broad-leaved and Korean pine mixed forest [26]. Currently, the area retains both undisturbed primary forest and secondary forest due to logging or fire. The main soil type in the area is mountain dark brown forest soil [27].

Forest plots were established at different successional stages. One plot represented an early successional stage (5.2 ha, established in 2005), one a mid-successional stage (5.2 ha, established in 2006), and one a late successional stage old-growth forest (40 ha, established in 2014). Plots were established according to the standard of the Forest Global Earth Observatory https://forestgeo.si.edu/ (accessed on 1 May 2014). Each plot was divided into 20 m × 20 m subplots, and in the same year after plot establishment, all woody stems with a diameter at breast height (DBH) of 1.0 cm or larger were spatially mapped, measured, identified to species, and labeled. Thereafter, the census was repeated every five years (approximately) following the first census methodology (Table 1).

Table 1. General information on forest plots at different successional stages in a temperate forest in the Changbai Mountain Natural Reserve, Northeast China.

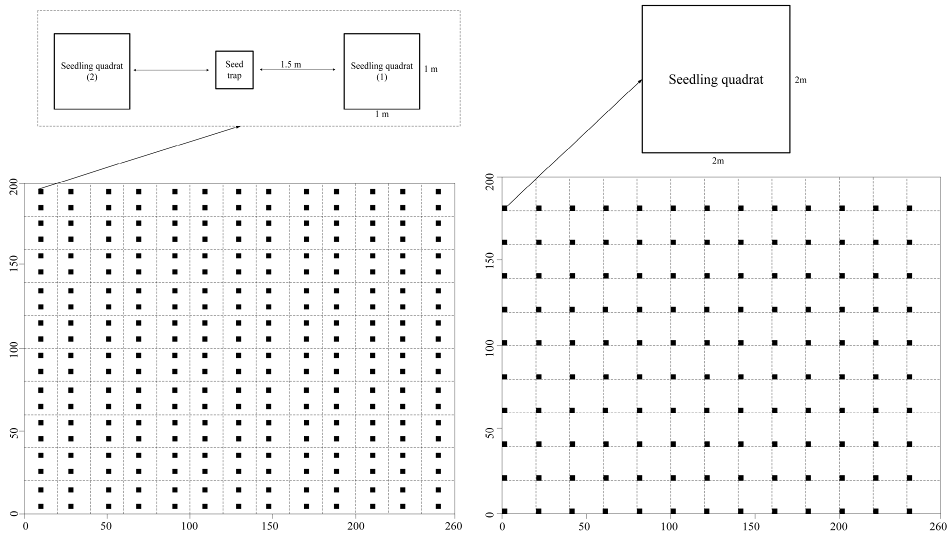
	Early Stage	Mid Stage	Late Stage
Land-use history	Clear-cut in 1930s	Selected-cut during 1930s–1940s	Undisturbed at least 200 years
Coordinates	42°19′10″ N, 128°07′50″ E	42°21′0″ N, 128°08′0″ E	42°20′1″ N, 127°54′47″ E
Elevation (m)	878	811	979
[min–max]	[873–887]	[807–817]	[958–998]
Plot size (ha)	5.2	5.2	40
[dimensions, m]	[260 × 200]	[260 × 200]	[800 × 500]
Establishment time	2005	2006	2014
Stem density ($N \cdot ha^{-1}$)	4450	2447	2509
DBH (cm, mean ± SE)	5.64 ± 0.05	8.45 ± 0.09	7.05 ± 0.04
Mean height (m, mean ± SE)	5.60 ± 0.03	7.20 ± 0.05	6.44 ± 0.02
Dominant species (sorted by BA)	*Betula platyphylla*, *Tilia amurensis*, *Fraxinus mandschurica*, *Populus davidiana*	*Tilia amurensis*, *Betula platyphylla*, *Abies nephrolepis*, *Quercus mongolica*	*Pinus koraiensis*, *Quercus mongolica*, *Tilia amurensis*, *Fraxinus mandschurica*

N, number; DBH, diameter at breast height; SE, standard error; BA, basal area at breast height. Source of measurement data: 2017 census data for early and mid-successional plots; 2019 census data for late-successional plot.

The distinct logging disturbances in the secondary forest plots have led to different successional stages at present [28], whereas the late successional plot is an original broad-leaved pine forest that has not experienced any anthropogenic disturbances [29]. Both of these secondary plots experienced logging disturbances around the same time. However, the early successional plot was clear-cut, while the mid-successional one was selectively logged, removing only some large-diameter timber. This difference in logging methods has further differentiated their current stages of community succession. Species composition is a critical indicator for determining community successional stages. Locally, the climax community is characterized by a broad-leaved pine forest, with dominant tree species including *Pinus koraiensis*, *Tilia amurensis*, *Fraxinus mandschurica*, and *Quercus mongolica*. Based on the dominant species composition, the three plots represented early, mid, and late stages of community succession.

2.2. Seedling Census Data and Mycorrhizal Types of Tree Species

In 2006, seedling census stations were evenly distributed in the subplots of early and mid-successional forest plots (Figure 1a). Each station consisted of a seed trap (0.5 m × 0.5 m) and two seedling quadrats (1.0 m × 1.0 m) at 1.5 m from the seed trap. There were two stations per subplot (130 subplots × 2 stations = 260 stations per secondary forest plot) [30]. In 2018, 88 seedling quadrats (2 m × 2 m) were set up at 20 m intervals in one corner of each subplot in the old-growth plot (Figure 1b) [31].



(a) (b)

Figure 1. Layout of seedling census quadrats in the forest plots at different stages of succession. (**a**) Distribution of seedling stations in forest plots at early and middle stages of succession. Each black square represents a seedling census station, and each station consists of a seed trap and two seedling quadrats. (**b**) Distribution of seedling quadrats in the forest plot at the late stage of succession, with each black square representing one seedling quadrat.

The first seedling census began in the summer of 2006, and the seedling quadrats were established. All tree seedlings (<1.0 cm DBH) within a quadrat were identified and labeled, and height (cm) was measured. All seedling quadrats were resurveyed each July to record the status (alive or dead) and growth (growth in height and root neck diameter) of all woody seedlings found in the previous census, and all newly recruited seedlings were identified, labeled, and measured. Seedling census data from 2010 to 2020 (no census data in 2016 and 2017 due to lack of manpower) were used for the early and mid-stage plots, and census data from 2018 to 2020 were used for the late-stage plot.

Mycorrhizal types for most tree species in the plots were determined according to the FungalRoot database [31] and other publications, except *Acer komarovii* [31–34]. Because most species in the genus *Acer* are AM species, *A. komarovii* was considered to be AM. In addition, although some species were associated with both AM and EcM fungi, given that EcM fungi are superior in defending against soilborne pathogens and protecting root tissues, those species were classified as EcM [10]. Mycorrhizal types of all tree seedling species are shown in Table S1 (with supporting references).

2.3. Neighborhood Variables

Only the effect of adult tree neighbors on seedling survival was considered. All adult large trees within a certain radius scale were divided into conspecific neighbors and heterospecific neighbors, with the focal seedling as the center.

For conspecific neighbors, the sum of the basal area ($BA = \pi r^2$) of all homogenous individuals divided by their respective distances to the focal seedling represents the conspecific neighbor tree density index (CI), which was calculated as follows:

$$\mathrm{CI(HI)} = \sum\nolimits_i^N \frac{\mathrm{BA}_i}{\mathrm{Distance}_i} \tag{1}$$

where N is the number of conspecific neighbors of a focal seedling, and distance is the distance between focal individual and its conspecific neighbor. The heterospecific neighbor density index (HI) was obtained similarly, by summing the values of BAs of heterospecific neighbors divided by the respective distances.

2.4. Abiotic Factors

2.4.1. Soil Characteristics

Two 500 g soil surface samples (0–20 cm in secondary forest plots and 0–10 cm in the old-growth forest plot) were randomly collected in each subplot and then mixed into one composite sample. Soil samples were air-dried and then analyzed for soil organic matter (OM), total nitrogen (TN), total phosphorus (TP), total potassium (TK), and pH according to methods recommended by the Soil Science Society of China (1999). Soil properties in the forest plots at different stages of succession are shown in Table S2.

2.4.2. Light Availability

Light availability in the understory was estimated using hemispherical photographs taken with a Nikon Coolpix 4500 camera body fixed with a Nikon FC-E8 Fisheye Converter lens (Japan). Each seedling plot was photographed from 1.5 m above the ground in 2013 and 2018. To indicate light availability, the canopy openness index (%) was generated using WinSCANOPY and XLScanopy programs [35]. Canopy openness in the forest plots at different successional stages in 2013 and 2018 is shown in Table S3.

2.5. Data Analyses

To ensure consistency of the time interval, only one-year survival of seedlings was considered in this study. To eliminate the influence of extreme values on the results, seedlings that appeared less than three times in the seedling census data were removed. To explore the roles of tree neighbors (i.e., DBH $\geq$ 1.0 cm) on seedling survival as precisely as possible, tree census data closest to seedling census time were used.

Distance of tree neighbors is also a factor that should not be ignored, particularly considering that large trees with a radius of 15–20 m have the most significant influence on seedling survival in temperate forests [36,37]. In this study, the tree neighborhood radius was set at 20 m, and all seedlings less than 20 m from the edge of a plot were excluded, because the neighbors of those focal individuals might be outside the plot. Effects of biotic neighbors with a radius of 5 m, 10 m, and 15 m were also determined, models with different neighborhood radii were compared using AIC and BIC. The best fit for most of the successional stages was a neighborhood radius of 20 m (Table S4). Thus, model results with a neighborhood radius of 20 m are the primary results reported in this study, and model results with the other neighborhood radii are presented in Figure S1.

Principal component analysis (PCA) was performed to reduce the collinearity of soil variables in the models. The top two principal component axes were ultimately selected, with the first principal component axis (PC1) representing the soil nutrient gradient and the second principal component axis (PC2) representing the soil acid–base gradient (Table 2).

Table 2. Loading scores of soil variables on the first two principal component (PC) axes in forest plots at different stages of succession.

Soil Variables	**Early Stage**		**Mid Stage**		**Late Stage**	
	PC1	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**
Organic matter (%)	0.586	−0.129	0.525	-	0.530	0.245
Total nitrogen (%)	0.488	0.311	0.462	0.108	0.567	-
Total phosphorus (%)	0.541	0.214	0.516	0.141	0.402	−0.438
Total potassium (%)	−0.355	0.538	−0.475	−0.114	−0.485	−0.131
pH	-	−0.742	0.139	−0.974	-	−0.855
Cumulative proportion (%)	36.0	58.7	48.0	67.8	57.4	80.5

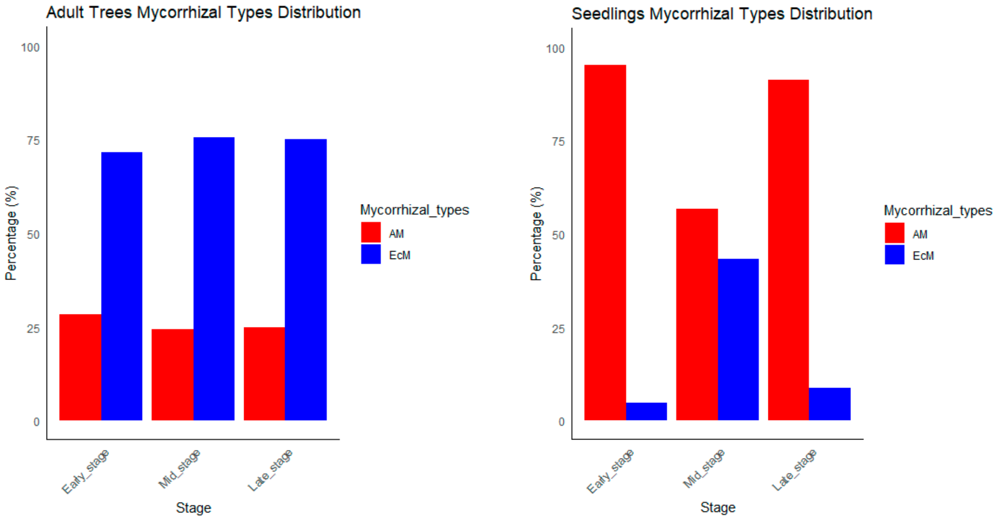
A series of generalized linear mixed effects models (GLMMs) were fit to investigate the effects of biotic and abiotic factors on seedling survival. The fixed effects of the GLMMs included individual size (log-transformed seedling height), CI, HI, canopy openness, and soil properties (PC1 and PC2), as well as the interaction effects of CI with abiotic factors.

All continuous effects variables were standardized by subtracting the mean and dividing by the standard deviation. Seedling species, quadrat size, and survey year were added as random effects to exclude the effects of factors such as spatial autocorrelation and climate on the model. Effects of biotic and abiotic factors on seedling survival were tested at community and mycorrhizal levels. The GLMM analyses were conducted utilizing the lme4 package via the glmer function, incorporating the Laplace Approximation method [38]. All computational processes were executed in R version 4.3.0 [39].

3. Results

The density and mycorrhizal affiliations of both seedlings and mature trees across successional stages were determined first, which established the basis for the primary investigation into the factors affecting seedling survival, highlighting the interplay between same-species tree neighbors and varying environmental conditions, as well as their interactions.

In the early successional stage, seedlings with AM fungi were dominant, with 95.2% of 29,526 seedlings in symbiosis with AM fungi (Figure 2b). This result suggests that, early in forest development, the environment was particularly suitable for AM fungi, possibly because of soil properties or the initial pioneer species. However, in adult trees in the early stage, EcM trees accounted for most of the basal area, with 71.7% of the total basal area of 30.75 $m^2 \cdot ha^{-1}$ (Table 3).



(**a**) (**b**)

Figure 2. Proportions of ectomycorrhizal (EcM) and arbuscular mycorrhizal (AM) (**a**) adult trees and (**b**) seedlings at different stages of forest succession.

Table 3. Density and mycorrhizal association (AM, arbuscular mycorrhizae; EcM, ectomycorrhizae) of adult trees (diameter at breast height ≥ 1 cm) and seedlings at different stages of forest succession.

Growth Stage	Mycorrhizal Types	Early Stage	Mid Stage	Late Stage
Adult trees	AM	8.70	8.61	11.07
	EcM	22.05	26.54	33.21
	Total	30.75	35.15	44.28
Seedlings	AM	28,097	6130	3885
	EcM	1429	4658	369
	Total	29,526	10,788	4254

For adult trees, density was based on basal area and is represented in $m^2 \cdot ha^{-1}$. For seedling density, individuals were counted.

In the mid-successional stage, the dominance of AM fungal seedling decreased, accounting for 56.8% of 10,788 seedlings, and seedling mycorrhizal types began to diversify (Figure 2b). This result suggests that adaptability of or advantages for EcM-associated seedlings increased in the intermediate phase of succession. Mature trees continued to be dominated by EcM associations, accounting for 75.5% of the total basal area of 35.15 $m^2 \cdot ha^{-1}$ (Table 3).

In the late successional stage, AM-associated seedlings were again dominant, accounting for 91.3% of the 4254 seedlings (Figure 2b). The increase indicates a fluctuating trend or perhaps changing environmental conditions that again favored AM associations. However, EcM associations remained dominant in mature trees in late succession, accounting for 75.0% of an expanded basal area of 44.28 $m^2 \cdot ha^{-1}$ (Table 3).

Although the primary research focus was on the multiple biotic and abiotic factors influencing young seedlings during succession, the constant EcM dominance in adult trees across all stages highlights the important and possibly crucial roles of EcM fungi in shaping successional environments (Figure 2a).

Seedling height was the main factor affecting seedling survival, and its effect was consistent between AM and EcM seedlings (Figures 3 and 4).

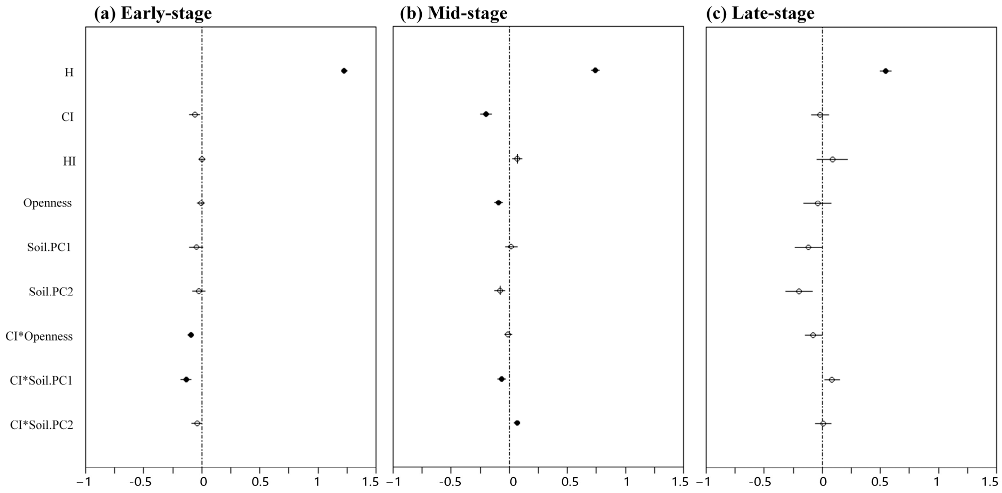


Figure 3. Estimated coefficients (mean ± SE) of biotic and abiotic variables on seedling survival for all species (community level) in forest plots at different stages of succession. (**a**) Early stage, (**b**) mid stage, and (**c**) late stage. A black dot indicates significance ($p < 0.05$); a dot with '+' indicates marginal significance ($p < 0.10$); and an open dot indicates no significance ($p \geq 0.10$).

At the community level, the strength and direction of biotic and abiotic effects on seedling survival depended on the stage of succession. In the early stage, the interactions between conspecific neighbor tree density (CI) and openness and soil PC1 significantly negatively affected seedling survival (Figure 3a). In the mid stage, CNDD effect and canopy openness were the main factors affecting seedling survival (Figure 3b). However, in the late stage, biotic and abiotic factors did not significantly affect seedling survival (Figure 3c).

Responses of different mycorrhizal types of seedlings to biotic and abiotic factors also varied across forest successional stages. In the early stage for AM seedlings, CNDD and its interaction with openness and soil PC1 were the main factors inhibiting seedling survival, whereas for EcM seedlings, soil PC1 and PC2 were the primary limiting factors (Figure 4a). In the mid stage, AM seedlings were mainly inhibited by CNDD and the environmental factors canopy openness and soil PC2. Survival of EcM seedlings was primarily limited by CNDD and its interaction with canopy openness (Figure 4b). In the late stage, there were no significant effects of biotic or abiotic factors on survival of either type of seedling (Figure 4c).

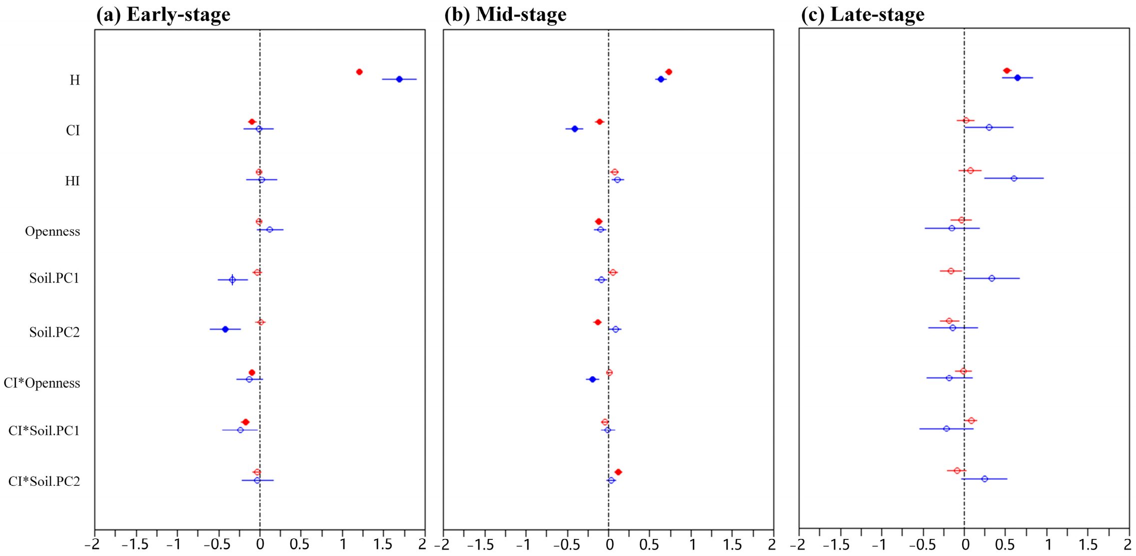


Figure 4. Estimated coefficients (mean ± SE) of biotic and abiotic variables on arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) seedling survival in forest plots at different stages of succession. (**a**) Early stage, (**b**) mid stage, and (**c**) late stage. Red circles indicate AM seedlings, and blue circles indicate EcM seedlings. Solid circles indicate significance ($p < 0.05$); circles with '+' indicate marginal significance ($p < 0.10$); and hollow circles indicate no significance ($p \geq 0.10$).

The strength of the CNDD effect, however, varied depending on the mycorrhizal type and the successional stage. The AM seedlings suffered stronger CNDD effects than those of EcM species in the early stage of succession (Figure 4a), but the trend reversed in the mid-successional stage (Figure 4b).

4. Discussion

4.1. Effects of Variables on Seedling Survival at the Community Level

Consistent with previous studies [2,4,17,40], we found significant CNDD effects in the temperate forest communities.

The transition of the CNDD effect from an interaction with environmental factors to a more direct effect as succession proceeded might be due to environmental limitations and differences in intensity of competition between individuals [20]. In the early successional stage, resources were well distributed and interspecific competition was strong, whereas in the late successional stage, intra- and interspecific competition weakened after the species occupied different ecological niches and stabilized.

Biotic factors also significantly affected seedling growth, which is also consistent with the results of previous studies [3]. Soilborne pathogens are significant positive factors affecting the CNDD mechanism [41]. However, in most cases, environmental variables (e.g., soil PC1) interacted with conspecific neighbor tree density to affect seedling survival, whereas the independent effects of environmental variables were relatively important in the mid-successional stage. The interaction between conspecific neighbor tree density and soil PC1 had a significant negative influence on seedling survival. In previous studies, increases in conspecific neighbor tree density increased the concentrations of specific soilborne pathogens, leading to mortality of conspecific seedlings [5,6].

4.2. Effects of Variables on Survival of Different Types of Mycorrhizal Seedlings

In this study, the CNDD effect on AM seedlings was the strongest in the early successional forest, which is consistent with the results of previous studies [10,42]. In addition, CNDD had the strongest effect on EcM seedlings in the mid-successional forest, which is a result supported by only a few studies [13]. Therefore, the strength of CNDD effects on AM and EcM seedlings varied with successional stage, which might be related to the proportions of adult trees of different mycorrhizal types in the community [12,43]. At the beginning of succession, the relatively high percentage of AM macrophytes might inhibit AM seedling survival by affecting the abundance of soil pathogens. In contrast, as succession progressed, the proportion of EcM adult trees within the community gradually increased, and thus, the proportion of soil pathogenic bacteria specific to the corresponding EcM species also increased, thereby inhibiting EcM seedling survival. Simultaneously, soil organic matter and nitrogen also increased during succession, which is consistent with results of previous studies [44–46]. Organic nutrients and nitrogen reserves in soil could promote the growth of EcM trees, and increases in soil nutrients could also promote the proliferation of soil pathogens to a certain extent, both of which would inhibit the survival of EcM seedlings. Because effects of the interactions between conspecific neighbor tree density and canopy openness and soil nutrients on different types of mycorrhizal seedlings were variable, AM- and EcM-associated tree species might mediate the influence of environmental factors [47].

Soil pH is an important factor that affects the intensity of plant–microbe interactions by altering microbial abundance and distribution [48]. Soil pH was negatively correlated with EcM seedling survival in this study, which is consistent with the results of a previous study in which soil pH was strongly negatively correlated with the dominance of EcM species in a community [49].

Soil factors also had different effects on seedlings of different mycorrhizal types. Compared with community-level studies, determining the differences in the responses of seedlings of different mycorrhizal types to different factors can help increase understanding of community-building mechanisms. Future studies need to consider how spatial and temporal dynamics of soil microorganisms regulate community-building mechanisms.

5. Conclusions

Conspecific negative density dependence and habitat filtering significantly affected seedling survival in different successional stages of a temperate forest in the Changbai Mountain Nature Reserve, Northeast China. At the community level, direct effects of CNDD and light were strongest in the mid-successional stage, but the interactions of CNDD with light and soil nutrients primarily suppressed seedling survival in the early successional stage. In the early successional plot, AM seedlings were mainly affected by CNDD and the interaction of CNDD with light and soil nutrients, whereas EcM seedlings were only directly affected by soil nutrients and pH. In the mid-successional plot, AM seedlings were affected by CNDD, light, and soil nutrients, and the intensity of those three factors was relatively similar. By contrast, EcM seedlings were mainly affected by CNDD in the mid-successional stage.

Unexpectedly, no significant effects of CNDD or habitat filtering were detected at the late successional stage, either at the community level or for seedlings of different mycorrhizal types. Factors such as soil moisture content (with excessively high levels potentially leading to seedling inundation and mortality) and terrain may be critical in determining seedling survival in the late successional stage. Furthermore, the relatively brief growing season in temperate forests might obscure certain species interactions, rendering them undetectable within the limited duration of the investigation. In future studies, the seasonal dynamics of light on seedling survival needs to be considered more carefully, and the spatial and temporal dynamics of soil microbes should be further explored to deepen understanding of the mechanisms of CNDD and the interactions between CNDD and the environment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14091919/s1, Table S1: Mycorrhizal types (AM, arbuscular mycorrhizae; EcM, ectomycorrhizae) of species of tree seedlings during forest succession; Table S2: Soil variables at different stages of forest succession (mean ± standard error); Table S3: Canopy openness (%) at different stages of forest succession (mean ± standard error); Table S4: AIC and BIC values for GLMMs of seedling survival at four neighborhood radii (5, 10, 15, 20 m) at different stages of forest succession. ∆AIC and ∆BIC are the values of AIC and BIC of models, respectively, with 5-, 10-, and 15-m neighborhood radii minus those with the 20-m neighborhood radius. Bold indicates the optimal scale of the model. AM, arbuscular mycorrhizae; EcM, ectomycorrhizae; Figure S1: Estimated coefficients (mean ± SE) of biotic and abiotic variables on seedling survival at different neighborhood radii in different stages of forest succession. Black circles represent the community level (i.e., all species combined), red circles represent AM seedlings, and blue circles represent EcM seedlings. Solid circles indicate significant effects ($p < 0.05$), while hollow circles indicate no significance ($p \geq 0.1$). In this context, "H" represents the height of seedlings, "CI" indicates the conspecific neighbors index, and "HI" denotes the heterospecific neighbors index. References [32,33,50,51] are cited in the supplementary materials.

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References

1. Bagchi, R.; Henrys, P.A.; Brown, P.E.; Burslem, D.F.R.P.; Diggle, P.J.; Gunatilleke, C.V.S.; Gunatilleke, I.A.U.N.; Kassim, A.R.; Law, R.; Noor, S.; et al. Spatial Patterns Reveal Negative Density Dependence and Habitat Associations in Tropical Trees. *Ecology* **2011**, *92*, 1723–1729. [CrossRef] [PubMed]
2. Comita, L.S.; Queenborough, S.A.; Murphy, S.J.; Eck, J.L.; Xu, K.; Krishnadas, M.; Beckman, N.; Zhu, Y. Testing Predictions of the Janzen–Connell Hypothesis: A Meta-analysis of Experimental Evidence for Distance- and Density-dependent Seed and Seedling Survival. *J. Ecol.* **2014**, *102*, 845–856. [CrossRef] [PubMed]
3. Johnson, D.J.; Condit, R.; Hubbell, S.P.; Comita, L.S. Abiotic Niche Partitioning and Negative Density Dependence Drive Tree Seedling Survival in a Tropical Forest. *Proc. R. Soc. B* **2017**, *284*, 20172210. [CrossRef] [PubMed]
4. LaManna, J.A.; Mangan, S.A.; Alonso, A.; Bourg, N.A.; Brockelman, W.Y.; Bunyavejchewin, S.; Chang, L.-W.; Chiang, J.-M.; Chuyong, G.B.; Clay, K.; et al. Plant Diversity Increases with the Strength of Negative Density Dependence at the Global Scale. *Science* **2017**, *356*, 1389–1392. [CrossRef]
5. Connell, J.H. On the Role of Natural Enemies in Preventing Competitive Exclusion in Some Marine Animals and in Rain Forest Trees. *Dyn. Popul.* **1971**, *298*, 298–313.
6. Janzen, D.H. Herbivores and the Number of Tree Species in Tropical Forests. *Am. Nat.* **1970**, *104*, 501–528. [CrossRef]
7. Klironomos, J.N. Feedback with Soil Biota Contributes to Plant Rarity and Invasiveness in Communities. *Nature* **2002**, *417*, 67–70. [CrossRef]
8. Mangan, S.A.; Schnitzer, S.A.; Herre, E.A.; Mack, K.M.L.; Valencia, M.C.; Sanchez, E.I.; Bever, J.D. Negative Plant–Soil Feedback Predicts Tree-Species Relative Abundance in a Tropical Forest. *Nature* **2010**, *466*, 752–755. [CrossRef] [PubMed]
9. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*; Academic Press: Cambridge, MA, USA, 2010; ISBN 0-08-055934-4.
10. Bennett, J.A.; Maherali, H.; Reinhart, K.O.; Lekberg, Y.; Hart, M.M.; Klironomos, J. Plant-Soil Feedbacks and Mycorrhizal Type Influence Temperate Forest Population Dynamics. *Science* **2017**, *355*, 181–184. [CrossRef]
11. Brown, A.J.; Payne, C.J.; White, P.S.; Peet, R.K. Shade Tolerance and Mycorrhizal Type May Influence Sapling Susceptibility to Conspecific Negative Density Dependence. *J. Ecol.* **2020**, *108*, 325–336. [CrossRef]
12. Jiang, F.; Lutz, J.A.; Guo, Q.; Hao, Z.; Wang, X.; Gilbert, G.S.; Mao, Z.; Orwig, D.A.; Parker, G.G.; Sang, W.; et al. Mycorrhizal Type Influences Plant Density Dependence and Species Richness across 15 Temperate Forests. *Ecology* **2021**, *102*, e03259. [CrossRef]
13. Jia, S.; Wang, X.; Yuan, Z.; Lin, F.; Ye, J.; Lin, G.; Hao, Z.; Bagchi, R. Tree Species Traits Affect Which Natural Enemies Drive the Janzen-Connell Effect in a Temperate Forest. *Nat. Commun.* **2020**, *11*, 286. [CrossRef]
14. Jevon, F.V.; De La Cruz, D.; LaManna, J.A.; Lang, A.K.; Orwig, D.A.; Record, S.; Kouba, P.V.; Ayres, M.P.; Matthes, J.H. Experimental and Observational Evidence of Negative Conspecific Density Dependence in Temperate Ectomycorrhizal Trees. *Ecology* **2022**, *103*, e3808. [CrossRef] [PubMed]
15. Dickie, I.A.; Schnitzer, S.A.; Reich, P.B.; Hobbie, S.E. Spatially Disjunct Effects of Co-Occurring Competition and Facilitation: Co-Occurring Competition and Facilitation. *Ecol. Lett.* **2005**, *8*, 1191–1200. [CrossRef] [PubMed]
16. Holík, J.; Janík, D.; Adam, D. Light Can Modify Density-dependent Seedling Mortality in a Temperate Forest. *J. Veg. Sci.* **2021**, *32*, e12992. [CrossRef]

17. LaManna, J.A.; Walton, M.L.; Turner, B.L.; Myers, J.A. Negative Density Dependence Is Stronger in Resource-rich Environments and Diversifies Communities When Stronger for Common but Not Rare Species. *Ecol. Lett.* **2016**, *19*, 657–667. [CrossRef]
18. Johnson, D.J.; Beaulieu, W.T.; Bever, J.D.; Clay, K. Conspecific Negative Density Dependence and Forest Diversity. *Science* **2012**, *336*, 904–907. [CrossRef]
19. Bruelheide, H.; Böhnke, M.; Both, S.; Fang, T.; Assmann, T.; Baruffol, M.; Bauhus, J.; Buscot, F.; Chen, X.-Y.; Ding, B.-Y.; et al. Community Assembly during Secondary Forest Succession in a Chinese Subtropical Forest. *Ecol. Monogr.* **2011**, *81*, 25–41. [CrossRef]
20. Yao, J.; Zhang, X.; Zhang, C.; Zhao, X.; Von Gadow, K. Effects of Density Dependence in a Temperate Forest in Northeastern China. *Sci. Rep.* **2016**, *6*, 32844. [CrossRef]
21. Burton, J.I.; Zenner, E.K.; Frelich, L.E.; Cornett, M.W. Patterns of Plant Community Structure within and among Primary and Second-Growth Northern Hardwood Forest Stands. *For. Ecol. Manag.* **2009**, *258*, 2556–2568. [CrossRef]
22. Lin, F.; Comita, L.S.; Wang, X.; Bai, X.; Yuan, Z.; Xing, D.; Hao, Z. The Contribution of Understory Light Availability and Biotic Neighborhood to Seedling Survival in Secondary versus Old-Growth Temperate Forest. *Plant. Ecol.* **2014**, *215*, 795–807. [CrossRef]
23. Kalacska, M.; Sanchez-Azofeifa, G.A.; Calvo-Alvarado, J.C.; Quesada, M.; Rivard, B.; Janzen, D.H. Species Composition, Similarity and Diversity in Three Successional Stages of a Seasonally Dry Tropical Forest. *For. Ecol. Manag.* **2004**, *200*, 227–247. [CrossRef]
24. Jiang, S.; Xing, Y.; Liu, G.; Hu, C.; Wang, X.; Yan, G.; Wang, Q. Changes in Soil Bacterial and Fungal Community Composition and Functional Groups during the Succession of Boreal Forests. *Soil Biol. Biochem.* **2021**, *161*, 108393. [CrossRef]
25. Wang, H.; Xu, J.; Sheng, L.; Ma, L.; Liu, X. 2020 Study on the Characteristics of Climate Change in Changbai Mountain National. *Arab. J. Geosci.* **2020**, *13*, 777. [CrossRef]
26. Yu, D.; Han, S. Ecosystem Service Status and Changes of Degraded Natural Reserves—A Study from the Changbai Mountain Natural Reserve, China. *Ecosyst. Serv.* **2016**, *20*, 56–65. [CrossRef]
27. Yue, Q.; Hao, M.; Li, X.; Zhang, C.; Gadow, K.; Zhao, X. Assessing Biotic and Abiotic Effects on Forest Productivity in Three Temperate Forests. *Ecol. Evol.* **2020**, *10*, 7887–7900. [CrossRef]
28. Cao, J.; Zhang, C.; Zhao, B.; Li, X.; Hou, M.; Zhao, X. Seedling Density Dependence Regulated by Population Density and Habitat Filtering: Evidence from a Mixed Primary Broad-Leaved Korean Pine Forest in Northeastern China. *Ann. For. Sci.* **2018**, *75*, 25. [CrossRef]
29. Liu, S.; Liao, J.-X.; Xiao, C.; Fan, X.-H. Effects of biotic neighbors and habitat heterogeneity on tree seedling survival in a secondary mixed conifer and broad-leaved forest in Changbai Mountain. *Chin. J. Plant Ecol.* **2016**, *40*, 711–722.
30. Miao, W.; Qiu-rong, L.; Dong-mei, X.; Bai-li, D. Effects of Soil Temperature and Soil Water Content on Soil Respiration in Three Forest Types in Changbai Mountain. *J. For. Res.* **2004**, *15*, 113–118. [CrossRef]
31. Soudzilovskaia, N.A.; Vaessen, S.; Barcelo, M.; He, J.; Rahimlou, S.; Abarenkov, K.; Brundrett, M.C.; Gomes, S.I.F.; Merckx, V.; Tedersoo, L. FungalRoot: Global Online Database of Plant Mycorrhizal Associations. *New Phytol.* **2020**, *227*, 955–966. [CrossRef]
32. Qin, J.; Geng, Y.; Li, X.; Zhang, C.; Zhao, X.; von Gadow, K. Mycorrhizal Type and Soil Pathogenic Fungi Mediate Tree Survival and Density Dependence in a Temperate Forest. *For. Ecol. Manag.* **2021**, *496*, 119459. [CrossRef]
33. Jiang, F.; Zhu, K.; Cadotte, M.W.; Jin, G. Tree Mycorrhizal Type Mediates the Strength of Negative Density Dependence in Temperate Forests. *J. Ecol.* **2020**, *108*, 2601–2610. [CrossRef]
34. Armand, N.; Shirvany, A.; Matinizadeh, M.; Khoshnevis, M. Seasonal changes in abundance of arbuscular mycorrhiza fungi of *Cerasus mahaleb* (L.) Mill. and their correlation with activity of some rhizosphere enzymes (case study: Chahartagh-e-Ardal). *Iran. J. For.* **2018**, *10*, Pe1–Pe12.
35. Yan, Y.; Zhang, C.; Wang, Y.; Zhao, X.; Gadow, K. Drivers of Seedling Survival in a Temperate Forest and Their Relative Importance at Three Stages of Succession. *Ecol. Evol.* **2015**, *5*, 4287–4299. [CrossRef]
36. Pu, X.; Jin, G. Conspecific and Phylogenetic Density-Dependent Survival Differs across Life Stages in Two Temperate Old-Growth Forests in Northeast China. *For. Ecol. Manag.* **2018**, *424*, 95–104. [CrossRef]
37. Pu, X.; Umaña, M.N.; Jin, G. Trait-Mediated Neighbor Effects on Plant Survival Depend on Life Stages and Stage-Specific Traits in a Temperate Forest. *For. Ecol. Manag.* **2020**, *472*, 118250. [CrossRef]
38. Bates, D.; Mächler, M.; Bolker, B.; Walker, S. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [CrossRef]
39. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2023; Available online: https://www.R-project.org/ (accessed on 13 June 2023).
40. Comita, L.S.; Muller-Landau, H.C.; Aguilar, S.; Hubbell, S.P. Asymmetric Density Dependence Shapes Species Abundances in a Tropical Tree Community. *Science* **2010**, *329*, 330–332. [CrossRef]
41. Laliberté, E.; Lambers, H.; Burgess, T.I.; Wright, S.J. Phosphorus Limitation, Soil-borne Pathogens and the Coexistence of Plant Species in Hyperdiverse Forests and Shrublands. *New Phytol.* **2015**, *206*, 507–521. [CrossRef]
42. Lambers, H.; Albornoz, F.; Kotula, L.; Laliberté, E.; Ranathunge, K.; Teste, F.P.; Zemunik, G. How Belowground Interactions Contribute to the Coexistence of Mycorrhizal and Non-Mycorrhizal Species in Severely Phosphorus-Impoverished Hyperdiverse Ecosystems. *Plant Soil* **2018**, *424*, 11–33. [CrossRef]
43. Tedersoo, L.; Bahram, M.; Zobel, M. How Mycorrhizal Associations Drive Plant Population and Community Biology. *Science* **2020**, *367*, eaba1223. [CrossRef] [PubMed]

44. Benavent-González, A.; Raggio, J.; Villagra, J.; Blanquer, J.M.; Pintado, A.; Rozzi, R.; Green, T.G.A.; Sancho, L.G. High Nitrogen Contribution by Gunnera Magellanica and Nitrogen Transfer by Mycorrhizas Drive an Extraordinarily Fast Primary Succession in Sub-Antarctic Chile. *New Phytol.* **2019**, *223*, 661–674. [CrossRef] [PubMed]
45. De Kroon, H.; Hendriks, M.; Van Ruijven, J.; Ravenek, J.; Padilla, F.M.; Jongejans, E.; Visser, E.J.W.; Mommer, L. Root Responses to Nutrients and Soil Biota: Drivers of Species Coexistence and Ecosystem Productivity: Root Responses and Ecosystem Productivity. *J. Ecol.* **2012**, *100*, 6–15. [CrossRef]
46. Liese, R.; Leuschner, C.; Meier, I.C. The Effect of Drought and Season on Root Life Span in Temperate Arbuscular Mycorrhizal and Ectomycorrhizal Tree Species. *J. Ecol.* **2019**, *107*, 2226–2239. [CrossRef]
47. Liu, X.; Burslem, D.F.R.P.; Taylor, J.D.; Taylor, A.F.S.; Khoo, E.; Majalap-Lee, N.; Helgason, T.; Johnson, D. Partitioning of Soil Phosphorus among Arbuscular and Ectomycorrhizal Trees in Tropical and Subtropical Forests. *Ecol. Lett.* **2018**, *21*, 713–723. [CrossRef]
48. Fierer, N.; Jackson, R.B. The Diversity and Biogeography of Soil Bacterial Communities. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 626–631. [CrossRef]
49. Lin, G.; Yuan, Z.; Zhang, Y.; Zeng, D.-H.; Wang, X. Dominant Tree Mycorrhizal Associations Affect Soil Nitrogen Transformation Rates by Mediating Microbial Abundances in a Temperate Forest. *Biogeochemistry* **2022**, *158*, 405–421. [CrossRef]
50. Iversen, C.M.; Powell, A.S.; McCormack, M.L.; Blackwood, C.B.; Freschet, G.T.; Kattge, J.; Roumet, C.; Stover, D.B.; Soudzilovskaia, N.A.; Valverde-Barrantes, O.J.; et al. Fine-Root Ecology Database (FRED): A Global Collection of Root Trait Data with Coincident Site, Vegetation, Edaphic, and Climatic Data, Version 2. Oak Ridge National Laboratory, TES SFA, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. FRED 3.0: The Fine-Root Ecology Database 3.0. 2018. Available online: https://roots.ornl.gov/ (accessed on 13 June 2023). [CrossRef]
51. Ren, J.; Fang, S.; Lin, G.; Lin, F.; Yuan, Z.; Ye, J.; Wang, X.; Hao, Z.; Fortunel, C. Tree Growth Response to Soil Nutrients and Neighborhood Crowding Varies between Mycorrhizal Types in an Old-Growth Temperate Forest. *Oecologia* **2021**, *197*, 523–535. [CrossRef]