

Distance-dependent effects of soil-derived biota on seedling survival of the tropical tree legume *Ormosia semicastrata*

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Abstract

Question: How do seed germination and subsequent seedling survival of *O. semicastrata* (Hance forma *litchiifolia* How) vary with respect to distance from parent trees and conspecific density in different types of tropical forest? Are there effects of soil biota on *O. semicastrata* that systematically depend on distance from parent trees and conspecific density? Do soil pathogens differently affect survival of *O. semicastrata* in different types of tropical forest?

Location: Tropical lowland rain forest and tropical montane rain forest in Jianfengling National Nature Reserve, Hainan Island, China.

Methods: Individual adult *O. semicastrata* trees were selected in lowland rain forest and montane rain forest. Soil was collected at a distance of 0–5 m or 15–20 m from the parent tree. Soil samples from each distance were combined into a bulk sample. Half of the soil sample was sterilized by autoclaving. Surface-sterilized seeds were then added to the soil material in shade-houses at both forests.

Results: Germination of *O. semicastrata* seeds at low- or high-seed density was barely affected by the sterilization procedure. In both forests, seedlings grown in non-sterilized soil collected close to parent trees had significantly higher mortality compared to those in sterilized soil. In contrast, seedling survival with soil collected far from parent trees was not affected by the soil sterilization procedure.

Conclusions: Host-specific pathogens concentrated in the soil around parent trees may regulate community structure of tropical trees at the stage of seedling development.

Keywords: Density effect; Lowland rain forest; Montane rain forest; Seed germination; Seedling survival; Soil pathogens.

Nomenclature: Anon. (1974–1999).

Introduction

Forest community structure and composition may be largely determined by early life history stages of tree species (Hille Ris Lambers & Clark 2003). Low seed germination rates and high mortality of seedlings may limit species distribution (Kobe 1999; Beckage & Clark 2003). Early reports indicated that individuals of tropical tree species are more regularly spaced than would be expected by a random process (Black et al. 1950; Pires et al. 1953). Janzen (1970) and Connell (1971) proposed that this pattern was the result of interacting effects of clumped seed dispersal patterns near parent trees and density- and distance-dependent effects of natural enemies on seeds, seedlings and saplings. In this hypothesis, seeds are available for germination and germinated seedlings survive better at a certain distance from parent trees. This distance effect can be explained by (i) lower disease transmission from parent trees to nearby offspring, and (ii) reduced impact of species-specific herbivores and pathogens.

Previous studies suggested that seedlings of many tree species exhibit higher mortality when seeds germinate at high density near parent trees (Augsburger 1983, 1984; Connell et al. 1984; Webb & Peart 1999; Gilbert et al. 2001). Based on a large-scale study in a tropical forest in Panama, Harms et al. (2000) found that seeds of a given tree species are less likely to become established seedlings if the seed density of that species is high. Taken together, these studies suggest that distance- or density-dependent mortality of seeds or seedlings around parent trees might frequently occur in forest ecosystems. However, the potential causes of this mortality pattern have been less frequently tested. For example, studies of distance- or density-dependent effects of soil pathogens are restricted to data

acquired from field investigations, and few manipulative experiments have been performed. The findings of these manipulative experiments indicate that soil pathogens have deleterious effects on seed germination and seedling survival of individual tree species. Studies in tropical forests showed that the presence of fungal pathogens resulted in significant losses of buried seeds (Dalling et al. 1998) and seedlings (Hood et al. 2004; Bell et al. 2006). Similarly, Packer & Clay (2000, 2003) found substantial impacts of soil pathogens on *Prunus serotina* seedlings in a temperate forest. These studies, performed in a single forest community, focused either on mortality at the stage of seed germination or at the stage of subsequent seedling development.

It is generally assumed that density-dependent mortality of seeds and seedlings is widespread in tropical forests, and this process can vary within an ecological gradient, as actions of natural enemies may differ in different habitat types. A number of field studies support the hypothesis that natural enemies drive density-dependent effects, resulting in spatial patterns of tree populations in specific habitat types. Density-dependent mortality was reduced in forest gaps for a range of tropical tree species (Augspurger 1983, 1984), and it was later hypothesized that lower soil humidity and higher light intensity reduced survival and action of pathogens (Augspurger 1990). However, the hypothesis of a “gap” effect for tree species in different types of tropical forests with different soil humidity and light intensity has not been supported by experimental data.

In this study, we performed experiments with the legume *Ormosia semicastrata*, which is a common canopy tree in both tropical lowland and montane rain forests in the Jianfengling National Natural Reserve on Hainan Island, China. It has a

similar density and importance value in the two forest types, allowing us to test whether density-dependent mortality varies with forest type. We specifically addressed the following questions: (1) How do seed germination and subsequent seedling survival of *O. semicastrata* vary with respect to conspecific density and distance from parent trees? (2) Are there effects of soil biota on *O. semicastrata* that systematically depend on distance from parent trees and conspecific density? (3) Do soil pathogens differently affect survival of *O. semicastrata* in different types of tropical forests?

Materials and Methods

Study sites

This study was conducted in Jianfengling National Nature Reserve (18°23'–18°52'N, 108°36'–109°05'E), located at the border between Ledong County and Dongfang County on Hainan Island, China (Fig. 1). The reserve has an area of approximately 20 170 ha, with three types of natural forest vegetation along an altitudinal gradient, i.e. tropical lowland rain forest, tropical montane rain forest and tropical cloud forest. These forests play an important role in maintenance of the local biodiversity. Our study was carried out in the lowland and montane rain forests of the reserve. Tropical lowland rain forest is a dominant vegetation type in this reserve, while the montane rain forest is characterized by the most complex forest structure and species composition among types of tropical forest. The meteorological characteristics in the two types of tropical forests are shown in Table 1.

Ormosia semicastrata is a legume in the Papilionaceae family that has the ability to establish root

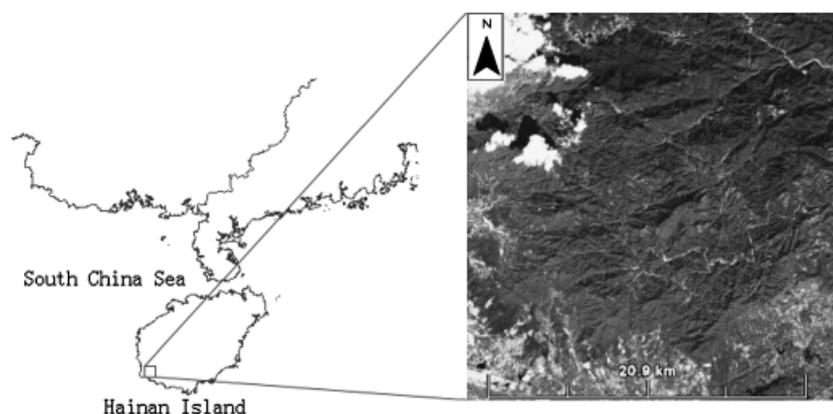


Fig. 1. Geographic location of Jianfengling National Nature Reserve.

Table 1. Meteorological characteristics of the two types of tropical forest at Jianfengling National Nature Reserve.

Meteorological characteristics	Lowland rain forest (200–700 m a.s.l.)	Montane rain forest (700–1200 m a.s.l.)
Temperature (°C/a)	22.9	19.7
Solar radiation [MJ/(m ² · a)]	5387.5	4938.6
Precipitation (mm/a)	1749	2447
Relative humidity (%)	75.0	84.4

nodule symbiosis with nitrogen-fixing bacteria (rhizobia). In the reserve, *O. semicastrata* is a canopy tree that is widely distributed in lowland and montane rain forests. It is generally assumed that *O. semicastrata* is a shade-intolerant tree species. Fruit production starts in August–September, and each fruit contains a single, cardinal-red seed, which reaches a diameter of approximately 9 mm. The seed dry mass is 0.16 to 0.19 g. Seeds of *O. semicastrata* have limited ability to disperse, thus seedlings are often densely distributed around parent trees.

Description of the experiment

Distance-dependent effects of soil-derived pathogens on *O. semicastrata* seed germination and subsequent seedling mortality were studied using an experimental approach. The experimental design and methodology were similar to those described in an earlier study conducted by Packer & Clay (2000). Two individual adult *O. semicastrata* trees were selected in lowland rain forest and montane rain forest (average \pm SD of tree diameters at breast height: 35.17 ± 6.95 cm in lowland rain forest and 28.13 ± 6.89 cm in montane rain forest; average \pm SD of crown radius: 2.88 ± 0.42 m in lowland rain forest and 2.58 ± 0.53 m in montane rain forest). Soil material at a depth of 0–30 cm was collected at two distances (0–5 m and 15–20 m) around these trees. For each forest, soil material collected at the same distance was combined into a bulk sample. One half of each bulk sample was autoclaved at 211°C for 1 h.

Plastic pots (15-cm diameter) were filled with the four different soil types (“0–5 m” and “15–20 m” distance; “sterilized” and “non-sterilized”). *O. semicastrata* seeds were collected at each study site, surface sterilized with a 10% sodium hypochlorite solution for 10 min, and thoroughly rinsed with distilled water. Seeds were then placed into the pots (two seeds per pot for the low-density treatment; four seeds per pot for the high-density treatment). The pots were watered regularly in order to keep soil

humidity constant during the course of the experiment. The experiment in each of the two forests was made up of eight treatments (four soil types; low and high density of seeds), and each treatment had 10 replicates (pots). Seed germination and seedling mortality were monitored every day. The experiments were conducted from late October 2006 to early June 2007 in shade-houses in both forests, providing approximately 20% of full sunlight.

Data analysis

For each experimental treatment, the proportion of seed germination and dead seedlings in each pot was calculated over the course of the experiment. Dead seedlings showed typical disease symptoms (leaf chlorosis, drooping leaves and leaves covered by powdery fungal hyphae). To test density-dependent effects on seedling survival, the seedling density in low-density treatments was confined to one seedling per pot by excluding replicates with two germinated seeds per pot. For the high-density treatments, replicates with at least two germinated seeds per pot were included in our analysis. Therefore, the average seedling density in high-density treatments ranged from 2.2 to 2.7 individuals per pot in the lowland rain forest experiment, and from 2.0 to 3.0 in the montane rain forest experiment. Values (percentage of germinated seeds and seedling survival) were log-transformed to meet assumptions of normality. Data were analyzed with a four-way analysis of variance (ANOVA) (distance from parent tree, conspecific density, sterilized soil and forest type), followed by a least-significant difference (LSD) test. All data analysis was performed with the software SPSS for Windows.

Results

Effect of soil sterilization on seed germination

Analysis of data from the experiment in the lowland rain forest did not result in any significant correlations between seed germination rate (percentage of germinated seedlings) and distance from parent trees, seed density or soil sterilization (Fig. 2a and b).

In the montane rain forest, seeds at high density in non-sterilized soil showed a higher germination rate for soil collected far (15–20 m) compared to close to parent trees (0–5 m) (LSD test, $P = 0.063$; Fig. 2c). Sterilization of soil, which was collected either far from or close to parent trees, had no

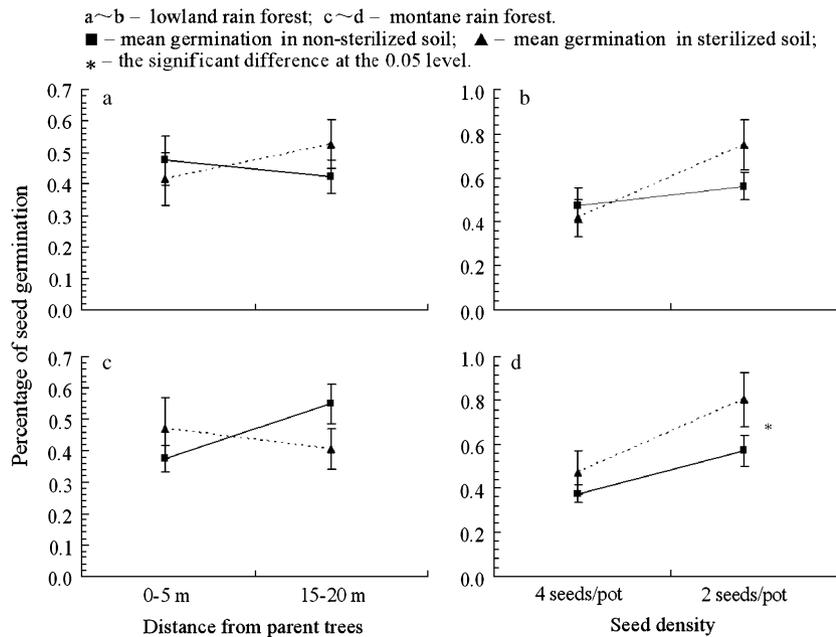


Fig. 2. Effects of distance from parent tree, seed density and soil sterilization on germination of *O. semicastrata* seeds in two types of tropical forest. Data were analysed with a four-way analysis of variance (ANOVA) (distance, density, sterilization and forest type); $F_{\text{density}} = 20.417$, degrees of freedom (df) = 1100, $P < 0.001$; $F_{\text{sterilization}} = 2.802$, $df = 1100$, $P = 0.098$; $F_{\text{density} \times \text{sterilization}} = 3.333$, $df = 1100$; $P = 0.071$; $F_{\text{density} \times \text{sterilization} \times \text{forest type}} = 4.066$, $df = 1100$; $P = 0.047$.

significant influence on seed germination (LSD test, $P = 0.382$ for soil collected close to parent trees and $P = 0.142$ for soil collected far from parent trees; Fig. 2c). Germination rates (\pm SE) in non-sterilized soil were $37.5 \pm 4.2\%$ at high-seed density and $57.1 \pm 7.1\%$ at low-seed density. With sterilized soil, germination rates were $47.2 \pm 9.7\%$ (high density) and $80.5 \pm 12.5\%$ (low density). There was marginally significant density-dependent germination in non-sterilized soil collected near parent trees (LSD test, $P = 0.057$; Fig. 2d). Sterilization of soil significantly enhanced the germination rate at low-seed density as compared with non-sterilized soil (LSD test, $P = 0.048$; Fig. 2d). Sterilization of soil, however, did not significantly affect the germination rate at high-seed density (LSD test, $P = 0.382$; Fig. 2d).

Effect of soil sterilization on seedling survival

The survival rate of *O. semicastrata* seedlings in non-sterilized soil was distance-dependent in both forest types (Fig. 3). Survival of seedlings grown in soil collected at a distance of 15–20 m was higher than in soil obtained close (0–5 m) to the parent trees (LSD test, $P = 0.017$ for lowland rain forest, Fig. 3a; $P = 0.009$ for montane rain forest, Fig. 3c). The percentage (\pm SE) of surviving seedlings in non-

sterilized soil was reduced at high-seedling density compared to low-seedling density in both forests ($65.3 \pm 8.2\%$ versus $85.7 \pm 14.3\%$ in the lowland rain forest; $60.0 \pm 10.0\%$ versus $83.3 \pm 16.7\%$ in the montane rain forest), although significant density-dependent seedling survival was only found in montane rain forest (LSD test, $P = 0.050$ in lowland rain forest, Fig. 3b; $P = 0.046$ in montane rain forest, Fig. 3d).

The effect of the soil sterilization treatments on seedling survival varied with distance from parent trees, as revealed by a significant interaction (ANOVA, $df = 162$, $F = 5.349$, $P = 0.025$). For seedlings grown at high density, sterilization of soil collected around parent trees significantly increased seedling survival in both forests (LSD test, $P = 0.038$ for lowland rain forest, Fig. 3a; $P = 0.006$ for montane rain forest, Fig. 3c), whereas sterilization of soil collected at a distance of 15–20 m did not significantly affect the survival rate (LSD test, $P = 0.940$ and $P = 0.493$ for lowland and montane rain forest, respectively; Fig. 3a and c). Soil sterilization also had no significant effect on survival of seedlings grown at low density (ANOVA, $df = 1, 9$, $F = 0.400$, $P = 0.398$ and $P = 0.404$ for lowland and montane rain forest, respectively; Fig. 3b and d).

At high-seedling density, the percentage (\pm SE) of surviving seedlings in non-sterilized soil collec-

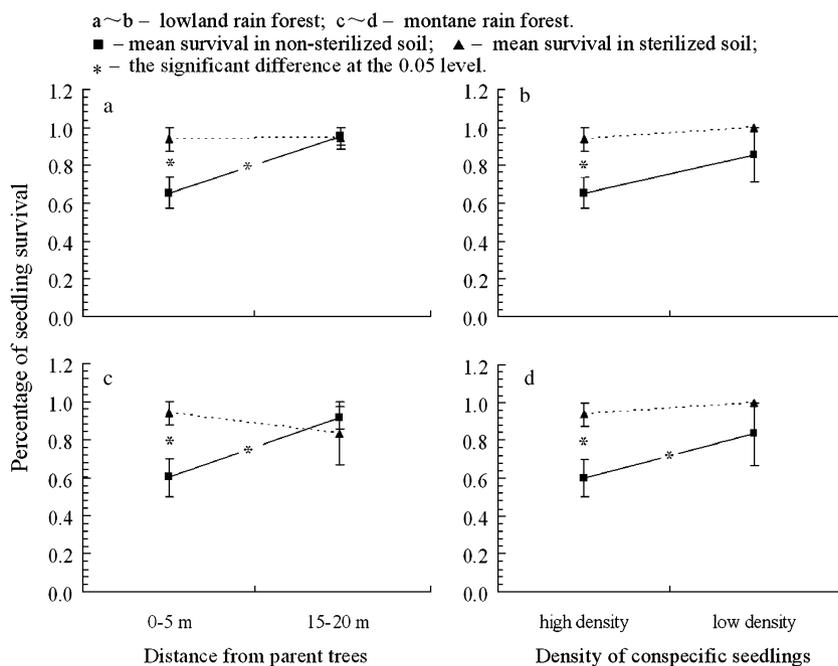


Fig. 3. Effects of distance from parent tree, conspecific density and soil sterilization on survival of *O. semicastrata* seedlings in two types of tropical forest. Data were analysed by a four-way analysis of variance (ANOVA) (distance, density, sterilization and forest type); $F_{\text{distance}} = 5.718$, degrees of freedom (df) = 162, $P = 0.021$; $F_{\text{density}} = 3.608$, $df = 162$, $P = 0.063$; $F_{\text{sterilization}} = 5.440$, $df = 162$, $P = 0.024$; $F_{\text{distance} \times \text{sterilization}} = 5.349$, $df = 1,62$; $P = 0.025$.

ted close to parent trees was similar in both forests ($65.3 \pm 8.2\%$ in lowland rain forest versus $60.0 \pm 10.0\%$ in montane rain forest, least-significant difference test, $P = 0.719$). Similarly, no significant differences between the two forests were found for seedlings grown at high density in non-sterilized soil collected at a distance of 15–20 m (LSD test, $P = 0.802$) and for seedlings at low density in non-sterilized soil collected close to parent trees (LSD test, $P = 0.861$).

Discussion

Mortality of buried seeds in natural communities is known to be caused by a wide range of herbivorous invertebrates and vertebrates, as well as various microbial pathogens (Baskin & Baskin 1998). It has been proposed that fungi are the most virulent pathogens for seeds and young seedlings (Blaney & Kotanen 2001). Crist & Friese (1993) studied effects of fungi on seed persistence of five species in a shrub-steppe system in Wyoming (USA), and reported that fungal attack strongly reduced survival of seeds. Fungicide treatments, which have been used in many studies, also indicate that fungal pathogens lead to increased seed

mortality (Lonsdale 1993; Dalling et al. 1998; Blaney & Kotanen 2001). In the present study, however, the experiment in the lowland rain forest did not reveal relationships between germination of *O. semicastrata* seeds and other tested variables, including soil sterilization. In the montane rain forest, the germination rate of seeds at high density in non-sterilized soil collected close (0–5 m) to parent trees was significantly lower than at a distance of 15–20 m from parent trees. For both distances, no significant difference between sterilized and non-sterilized soil was found, however. Thus, germination of *O. semicastrata* seeds was apparently not affected by external soil pathogens during the experiment. This does not exclude the possibility that internal pathogens within seeds affected germination of *O. semicastrata* (seed-borne pathogens could not be eliminated by the seed surface sterilization procedure). Furthermore, the large protein-rich seeds of *O. semicastrata* have thick seed coats. Similar to other tropical legumes (Janzen et al. 1986), *O. semicastrata* seeds apparently accumulate a set of preformed defence substances, which protect the seedlings from pathogen attack during the germination stage.

In contrast to the results for seed germination, the findings from both study sites in our experiment

clearly show that non-sterilized soil collected close (0–5 m) to parent trees had deleterious effects on subsequent seedling survival. Conspecific density also exerted a powerful influence on survival of seedlings grown in non-sterilized soil collected close to parent trees in both study sites. These findings are consistent with the Janzen-Connell hypothesis (Janzen 1970; Connell 1971). Reduced density-dependent seedling survival in areas close to adults, or in areas of high seedling density has been reported for a range of tropical tree species (Augspurger 1983, 1984; Connell et al. 1984; Webb & Peart 1999; Gilbert et al. 2001), suggesting rapid propagation and transmission of host-specific soil pathogens in such areas (Augspurger 1983).

Previous studies on tropical forests suggested that soil pathogens are important biotic factors causing density-dependent mortality at the seedling stage (Ashton 1988; Hood et al. 2004). For example, seedlings in untreated plots experienced eight-times higher mortality compared to those in plots treated with a fungicide. Seedling survival in fungicide-treated plots was not affected by seedling density, but survival of seedlings in untreated plots was higher at low density (Bell et al. 2006). This is consistent with our data from both forest types. *O. semicastrata* seedlings at high density in non-sterilized soil from close to parent trees had significantly higher mortality compared to seedlings in sterilized soil, whereas seedling survival with soil collected at a distance of 15–20 m from parent trees was not affected by the soil sterilization procedure. These findings are consistent with the hypothesis that host-specific pathogens are concentrated in soil around parent trees (Packer & Clay 2003). Seedling survival in sterilized soil was similar at either high or low density, suggesting that competition for resources between seedlings is not a major factor affecting seedling survival in this species (Wright 2002). Soil sterilization can change nutrient availability, resulting in a flush of nutrients and positive effects on plant growth (Troelstra et al. 2001). Reinhart et al. (2005) used a small portion of non-sterile soil as an inoculum for sterilized soil to minimize these effects. The same study also showed that results from fungicide treatments correlated with those from soil sterilization treatments. In agreement with these reports, the data from our soil sterilization experiments strongly support the hypothesis that soil-derived pathogens are responsible for distance-dependent survival of *O. semicastrata* seedlings. Future work is required to identify these pathogens and to characterize their effects on host and non-host plants.

Dynamics of interactions between plants and soil biota have been reported for several species (Packer & Clay 2000; Klironomos 2002; Hood et al. 2004). A study suggested that the relationship between the grasses *Panicum* and *Anthoxanthum* may be influenced by the density of pathogenic *Pythium* damping-off fungi (Bever 1994). Similarly, Reinhart et al. (2005) found that *Prunus serotina* seedlings were negatively affected by soil biota associated with 22 *P. serotina* populations located throughout the eastern USA. Previous reports indicated that the negative effect of soil biota on plants is host-specific (Van der Putten & Peters 1997; Klironomos 2002). In sand dunes, Van der Putten et al. (1993) found that invasion of root pathogens into the root zone of susceptible marram grass (*Ammophila arenaria*) led to a decrease in the abundance of this plant, resulting in its replacement by a resistant grass, *Festuca rubra*. Packer & Clay (2000) used *Pythium* isolates from *P. serotina* seedlings for inoculation experiments and found seedling mortality of *P. serotina* of 100%, whereas many other species, at least in part, were resistant to these *Pythium* isolates. Based on these reports and the data of our study, we expect that host-specific pathogens, especially soil-derived fungi, play an important role in regulating populations of *O. semicastrata* and other trees in tropical forest communities.

Although soil humidity and light intensity were obviously different between the two forests under natural conditions, density-dependent mortality of *O. semicastrata* seedlings was similar in both forests. The soil was regularly watered to maintain soil humidity approximately constant during the experiment. In other words, soil humidity was not different in the shade-houses at the two study sites. Moreover, seedlings were exposed to approximately 20% of full sunlight in both forests. It would be of interest to test the effect of pathogens on *O. semicastrata* under experimental conditions that simulate the environmental conditions of lowland and montane rain forests. Long-term studies are in progress to characterize the requirements for tree development at both study sites.

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References

- Anon. (Editorial Committee of the Chinese Academy of Sciences for Flora of China) 1974-1999. *Flora of China*. Science Press, Beijing, CN.
- Ashton, P.S. 1988. Dipterocarp reproductive biology. In: Lieth, H. & Werger, M.J.A. (eds.) *Tropical rain forest ecosystems*, pp. 220–240. Elsevier, Amsterdam, NL.
- Augsburger, C.K. 1983. Seed dispersal of the tropical tree, *Platydictyon elegans*, and the escape of its seedlings from fungal pathogens. *Ecology* 65: 1705–1712.
- Augsburger, C.K. 1984. Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps and pathogens. *Ecology* 65: 1705–1712.
- Augsburger, C.K. 1990. Spatial patterns of damping-off disease during seedling recruitment in tropical forests. In: Burdon, J. & Leather, S. (eds.) *Pests, pathogens and plant communities*, pp. 131–143. Blackwell Scientific, Oxford, GB.
- Baskin, C.C. & Baskin, J.M. 1998. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. Academic Press, San Diego, CA, US.
- Beckage, B. & Clark, J.S. 2003. Seedling survival and growth of three forest tree species: the role of spatial heterogeneity. *Ecology* 84: 1849–1861.
- Bell, T., Freckleton, R.P. & Lewis, O.T. 2006. Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecology Letters* 9: 569–574.
- Bever, J. 1994. Feedback between plants and their soil communities in an old-field community. *Ecology* 75: 1965–1977.
- Black, G.A., Dobzhansky, T. & Pavan, C. 1950. Some attempts to estimate species diversity and population density of trees in Amazonian forests. *Botanical Gazette* 111: 413–425.
- Blaney, C.S. & Kotanen, P.M. 2001. Effects of fungal pathogens on seeds of native and exotic plants: a test using congeneric pairs. *Journal of Applied Ecology* 38: 1104–1113.
- Connell, J.H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: den Boer, P.J. & Gradwell, G. (eds.) *Dynamics of populations*. pp. 298–312. Centre for Agriculture Publishing and Documentation, Wageningen, NL.
- Connell, J.H., Tracey, J.G. & Webb, L.J. 1984. Compensatory recruitment, growth, and mortality as factors maintaining rain forest tree diversity. *Ecological Monographs* 54: 141–164.
- Crist, T.O. & Friese, C.F. 1993. The impact of fungi on soil seeds: implication for plants and granivores in a semiarid shrub steppe. *Ecology* 74: 2231–2239.
- Dalling, J., Swaine, M. & Garwood, N. 1998. Dispersal patterns and seed bank dynamics of pioneer trees in moist tropical forest. *Ecology* 79: 564–578.
- Gilbert, G.S., Harms, K.E., Hamil, D.N. & Hubbell, S.P. 2001. Effects of seedling size, El Niño drought, seedling density, and distance to nearest conspecific adult on 6-year survival of *Ocotea whitei* seedlings in Panamá. *Oecologia* 98: 100–108.
- Harms, K.E., Wright, J.S., Calderón, O., Hernández, A. & Herre, E.A. 2000. Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature* 404: 493–495.
- Hille Ris Lambers, J. & Clark, J.S. 2003. Effects of dispersal, shrubs, and density-dependent mortality on seed and seedling distributions in temperate forests. *Canadian Journal of Forest Research* 33: 783–795.
- Hood, L.A., Swaine, M.D. & Mason, P.A. 2004. The influence of spatial patterns of damping-off distance and arbuscular mycorrhizal colonization on tree seedling establishment in Ghanaian tropical forest soil. *Journal of Ecology* 92: 816–823.
- Janzen, D.H. 1970. Herbivores and the number of tree species in tropical forests. *American Naturalist* 104: 501–528.
- Janzen, D.H., Ryan, C.A., Liener, I.E. & Pearce, G. 1986. Potentially defensive proteins in mature seeds of 59 species of tropical Leguminosae. *Journal of Chemical Ecology* 12: 1469–1480.
- Klironomos, J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417: 67–70.
- Kobe, R.K. 1999. Light gradient partitioning among tropical tree species through differential seedling growth and mortality. *Ecology* 80: 187–201.
- Lonsdale, W. 1993. Losses from the seed bank of *Mimosa pigra*: soil micro-organisms vs. temperature fluctuations. *Journal of Applied Ecology* 30: 654–660.
- Packer, A. & Clay, K. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature* 404: 278–281.
- Packer, A. & Clay, K. 2003. Soil pathogens and *Prunus serotina* seedling and sapling growth near conspecific trees. *Ecology* 84: 108–119.
- Pires, J.M., Dobzhansky, T. & Black, G.A. 1953. An estimate of the number of species of tree in an Amazonian forest community. *Botanical Gazette* 114: 467–477.
- Reinhart, K.O., Royo, A.A., Van Der Putten, W.H. & Clay, K. 2005. Soil feedback and pathogen activity in *Prunus serotina* throughout its native range. *Journal of Ecology* 93: 890–898.
- Troelstra, S.R., Wagenaar, R., Smant, W. & Peters, B.A.M. 2001. Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. *New Phytologist* 150: 697–706.

- Van Der Putten, W.H. & Peters, B.A.M. 1997. How soilborne pathogens may affect plant competition. *Ecology* 78: 1785–1795.
- Van der Putten, W.H., Van Dijk, C. & Peters, B.A.M. 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature* 362: 53–56.
- Webb, C.O. & Peart, D.R. 1999. Seedling density promotes coexistence of Bornean rain forest trees. *Ecology* 80: 2006–2017.
- Wright, S.J. 2002. Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* 130: 1–14.

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