

Spatial patterns of sapling mortality in a moist tropical forest: consistency with total density-dependent effects

Eduardo Velázquez^{1,2}, Martin Kazmierczak¹ and Thorsten Wiegand^{1,3}

¹Dept of Ecological Modelling, Helmholtz Centre for Environmental Research - UFZ, Permoserstrasse 15, DE-04318, Leipzig, Germany.

²Present address for EV, Depto de Biología, Centro Universitario de Coyhaique, Univ. de Magallanes, Coyhaique, Chile.

³German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, DE-04103 Leipzig, Germany.

Corresponding author: E. Velázquez, Dept of Ecological Modelling, Helmholtz Centre for Environmental Research - UFZ, Permoserstrasse 15, DE-04318, Leipzig, Germany. E-mail: eduardo.velazquez@ufz.de

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Abstract

Density-dependent mortality (DDM) is major driver of species coexistence in tropical forests. We assessed the occurrence and strength of this mechanism among saplings (i.e. trees with a diameter of 1–4 cm) in the moist tropical forest of Barro Colorado Island (BCI), Panama, over six inter-census periods (26 years), by using spatial point pattern analysis. We considered conspecific density-dependent mortality, but also total DDM (i.e. the effects of conspecific and heterospecific neighbours all together) upon saplings of all species (i.e. at the community level), and on saplings of light-demanding and shade-tolerant ones, separately (i.e. at the light-guild level). We also analysed the effects of conspecific DDM at the community and light-guild levels. Conspecific density-dependent mortality affected a lower proportion of species than was to be expected. Its strength was not significantly related with species abundance, and it had relatively weak effects at the community and light guild levels. Conversely, we detected a significant spatial signature of total DDM among all saplings, and among saplings of the light-demanding and shade-tolerant species, but its strength showed pronounced temporal variation. Total DDM among light-demanding saplings was stronger than among shade-tolerant ones and its effects were particularly marked 10–15 years after the occurrence of unusually severe droughts associated with El Niño events. Our study indicates that conspecific DDM is relatively unimportant among saplings in comparison with total DDM at community and light-guild levels. This finding contrasts strongly with the results for seedlings, where conspecific DDM was constituted the dominant process. The pronounced temporal variations observed in the occurrence and strength of total DDM among saplings indicate that time-varying events associated with climatic disturbances such as canopy openings or episodes of high recruit emergence might be important drivers of this process. They also emphasize the importance of considering time frames longer than one decade to study density-dependent effects among saplings in tropical forests.

(Introduction)

Understanding the coexistence of species in highly diverse ecosystems such as tropical forests poses one of the biggest challenges in community ecology (Wright 2002). In the early stages of tree development density-dependent mortality (hereafter; DDM) is generally believed to constitute an important factor in the maintenance of this diversity (Comita et al. 2014). According to the density-dependent prediction of the *Janzen-Connell hypothesis* (Janzen 1970, Connell 1971), conspecific density-dependent mortality keeps the density of each species in check and has the potential to increase species evenness and richness in the community. This process stems primarily from the proliferation of species-specific herbivores and pests in high-density patches of conspecific individuals (Hille Ris Lambers et al. 2002, Comita et al. 2010).

However, mortality of saplings may also be affected by the density of all neighbours and not only of conspecifics (Carson et al. 2008). In this case, the effect of a conspecific or heterospecific neighbour on the survival of a focal sapling would be mediated by competition for limiting resources (such as light) and would depend upon its proximity and size, rather than its species identity (Hubbell 2006). Under this *species-blind* or total density dependent mortality, the survival of focal saplings of a particular species will be determined more by life-history characteristics than by the species identity of neighbours (*ecological equivalence hypothesis*; Uriarte et al. 2005). In tropical forests, where light is one of the most limiting resources (Rüger et al. 2009), the light guild of the neighbours may constitute an important driver of sapling mortality in high-density areas (Carson et al. 2008). In particular, total DDM is expected to be greater among saplings of light-demanding species, which tend to occur together in gaps and compete strongly for the available light (Hubbell et al. 1999), than among saplings of shade-tolerant species, which are more broadly located in the understory (Uriarte et al. 2004).

Evidence of DDM has been detected mainly among seedlings (i.e. woody individuals < 1 cm diameter at breast height), in tropical forests of Panama (Comita and Hubbell 2009, Comita et al. 2010), Costa Rica (Kobe and Vriesendorp 2011), Ecuador (Metz et al. 2010), French Guiana (Paine et al. 2012), Peru (Álvarez-Loayza and Terborgh 2011) and Borneo (Webb et al. 2006). These and other studies have accumulated strong evidence that seedling survival is negatively affected mainly by conspecific neighbours (Comita et al. 2014, Lebrija-Trejos et al. 2014). The findings of studies on density-dependent mortality (and growth) of saplings and larger trees, however, are different. For instance, analysis of the first 13 years of available data (1982-1995) from the large-scale permanent forest plot of Barro Colorado Island (BCI), Panama, showed that overall stem density was the strongest predictor of negative effects on focal plant survival, followed in importance by conspecific density; research in this sense also revealed that conspecific DDM had a greater effect upon the rare species than on the most abundant ones (Hubbell et al. 2001). Moreover, sapling survival in the BCI plot was negatively affected by all neighbours, without distinction between conspecific and heterospecific ones, for 34 of 50 species (Uriarte et al. 2005). These findings, together with those recently reported for other tropical

(Luo et al. 2012) and temperate (Piao et al. 2013) forests, indicate that, in contrast to seedlings, sapling mortality mostly involves total density-dependent effects.

However, while DDM among seedlings can be detected in a relatively straightforward manner, the detection of this process among saplings poses several difficulties. Firstly, while conspecific DDM of seedlings may occur within a few months, that of saplings takes several years to become evident (Zhu et al. 2010). Secondly, saplings can remain in the understory of tropical forests for over two decades, and their mortality usually varies over time as a consequence of episodes of high recruit emergence (Carson et al. 2008), periodic outbreaks of specialist insects (Gilbert et al. 2005) or unusually severe droughts imposed by El Niño events (Condit et al. 2004). We therefore expect the occurrence of conspecific and total DDM among saplings to be consistent, but its strength will likely vary over time. Such temporal variations cannot be detected if these two processes are analysed over time frames of less than 15 years (Metz et al. 2010) but to date, few datasets span longer time periods (Feeley et al. 2011). The data from BCI, where all tree individuals > 1 cm DBH (diameter at 1.3 m above ground) have been mapped and re-censused every five years since 1985 (Hubbell et al. 2005), provide a unique opportunity to conduct long-term analyses of conspecific and total DDM among saplings to assess potential variability in the strength of these processes.

Moreover, the data provided by two subsequent censuses in large-scale permanent forest plots such as that of BCI include the spatial location (x, y) of each individual tree, its species identity and its status (i.e. surviving or dead). In this type of datasets, called *qualitatively marked point patterns*, we can detect the signature of DDM in the spatial distribution of trees with the use of recent techniques of spatial point pattern analysis (Wiegand and Moloney 2014). These techniques are complementary to the individual-based neighbourhood analyses that have been previously applied for this purpose (e.g. Uriarte et al. 2004, Comita et al. 2010), but they enable determination of the continuous range of spatial scales at which density-dependent effects occurs (e.g. Velázquez et al. 2014). The *spatial signature* of conspecific and total DDM, characterized by significantly higher pre-mortality sapling density around dead saplings than around surviving saplings at a specific range of scales, is very useful with regard to determining the underlying mechanisms of these processes (Luo et al. 2012, Piao et al. 2013).

In the present study we used spatial point pattern analysis to assess the occurrence and strength of conspecific and total density-dependent mortality among saplings in the moist tropical forest of the Forest Dynamics Plot at Barro Colorado Island (BCI), Panama. We performed three types of analyses. First, for each species, we analysed whether sapling mortality depends on the neighbourhood density of conspecific saplings (i.e. conspecific or population level DDM). Thus, we focused on the effects of conspecific sapling density upon sapling survival (i.e. the density-dependent mechanism of the *Janzen Connell hypothesis*), leaving the effects of the proximity of larger trees (i.e. the distance-dependent mechanism) for forthcoming studies. Second, we pooled all saplings of the community and those of light-demanding and shade-tolerant species (i.e. total DDM at community and light-guild levels, respectively) to investigate

whether sapling mortality depends on the neighbourhood density of all saplings regardless of species. Furthermore, it is important to note that the spatial signature of total DDM among saplings may arise in two different cases; first, if conspecific DDM is generally weak although species are arranged in multispecies clumps where total DDM is operating, and second, if conspecific DDM is occurring in a large proportion of species due to conspecific clumping. To differentiate between these two cases we performed a third type of analyses by pooling the results of conspecific DDM for all species (i.e. at the community-level) and for all light-demanding and all shade-tolerant species, separately (i.e. at the light-guild level). These analyses differ with those of total DDM in the way at which the neighbourhood density of a focal individual was estimated; for total DDM at the community or light-guild level we considered both, conspecific and heterospecific neighbours, whereas for conspecific DDM at the community or light guild level we only considered conspecific neighbours.

Based on the analyses at these three levels, which we performed for the six available inter-census periods and for the entire 27-year period between 1982 and 2010, we hypothesized that: (1) conspecific DDM will occur among saplings, but its spatial signature and strength will vary among inter-census periods; (2) conspecific DDM will have a greater effect on saplings of the rare species than upon saplings of the most abundant ones, and therefore, it will be negatively and significantly related to species abundance; (3) total DDM at the community level will occur among saplings but its spatial signature and strength will vary among inter-census periods; (4) total DDM at the light-guild level will be stronger among saplings of light-demanding species than among those of shade-tolerant ones, and (5) total DDM will be stronger than conspecific DDM at the community and light-guild levels.

Material and Methods

Study area and data collection

The study was conducted in the 50-ha Forest Dynamics Plot of Barro Colorado Island (BCI), Panama (9° 9' N; 79° 51' W), at 120-160 m a.s.l., where rainfall averages 2600 mm/year and is seasonal, with a peak from April to November (Hubbell et al. 2004). This plot is occupied by a well-studied semi-deciduous moist tropical forest in which all woody plant individuals ≥ 1 cm DBH were mapped, measured and identified to species in 1982 and every five years from 1985 to 2010 (Condit 1998; Hubbell et al. 2005). In this forest there is also a large number of rare and light-demanding species (Hubbell et al. 2001) and mortality is remarkably high at all tree life stages (Condit et al. 2004). The canopy is less closed than in other moist and wet tropical forests and tree-fall gaps and canopy-openings have been relatively frequent over the 27-year study period (Lobo and Dalling 2014). These disturbances have occurred particularly in years following El Niño events (e.g. 1985 and 2000), when dry seasons have been unusually severe (Hubbell 2004).

We defined saplings as all woody individuals with a 1-4 cm DBH (Uriarte et al. 2004) and used the position data (x, y coordinates) of all saplings in this plot from the eight available censuses. We tested the occurrence of conspecific and total DDM over the 1982-1985, 1985-1990, 1990-1995, 1995-2000, 2000-2005 and 2005-2010 periods, denoted as the first, second, third, fourth, fifth and sixth inter-census periods, respectively, and over the entire 1982-2010 period. In each of them we consider all saplings which survived up to the last year of the period (many of them recruited in former censuses) as *surviving* and all saplings that died as *dead*. We classified species as light-demanding and shade-tolerant according to the index of Comita et al. (2007) which is based on the 95% quartile of sapling growth rates. This classification is closely related to the growth-mortality trade-off and provides a practical differentiation among the tree species at both ends of the continuum of light requirements existing in this plot (Rüger et al. 2009).

Spatial signature and strength of density- dependent mortality (H1-4)

We tested hypotheses 1 to 4 by performing separate point pattern analyses over each inter-census period, for the following data sets; (i) conspecific saplings (i.e. conspecific DDM at the species level), (ii) all saplings summed over species (i.e. total DDM at the community level), and (iii) all light-demanding and (iv) shade-tolerant saplings (i.e. total DDM at the light-guild level). For analysis (i) we considered only species with ≥ 70 saplings in a given period because, with fewer saplings, stochasticity precludes meaningful spatial point pattern analysis (Wiegand et al. 2007). This criterion resulted in the inclusion of 114-136 species out of the 320 found in BCI, and 92 - 96% of the sapling community, depending on the inter-census period, and on the inclusion of 140 species (98% of the sapling community) for the 1982-2010 period.

Over each period and for each of the groupings (i) to (iv), we created datasets consisting of a point pattern comprising the spatial positions of the saplings in the BCI plot and their label *surviving* or *dead*. We contrasted the summary statistics of the observed datasets against those arising from Monte Carlo simulations of the null model of random mortality (Goréaud and Pélissier 2003). This null model maintains the observed spatial positions of the saplings but randomly shuffles their mark *dead* (Wiegand and Moloney 2014). The spatial patterns generated by this null model showed no signature of density-dependent effects.

To detect the range of spatial scales over which DDM occurred (i.e. its *spatial signature*), we used the test statistic $DDM(r)$ which has been specifically designed for this purpose (Wiegand and Moloney 2014: their section 4.4.1.3). This summary statistic is based on the partial O -ring statistics $O_{1,1+2}(r)$ and $O_{2,1+2}(r)$ that estimate the mean density of (pre-mortality) saplings in a ring of radius r and width dw centred around the *dead* (subscript 1) and *surviving* (subscript 2) saplings, respectively. The $DDM(r)$ summary statistic then compares the mean sapling density around dead saplings with that found around surviving ones (Velázquez et al. 2014). Thus, under DDM, dead saplings possess higher pre-mortality density of neighbours at distance r than surviving saplings. To obtain a summary statistic that is independent

from the pre-mortality density of saplings we divided the O-ring statistics by the intensity $\lambda_{1+2} = n_{1+2}/A$ (n_{1+2} is the number of pre-mortality saplings and A the area of the plot):

$$DDM(r) = [O_{1,1+2}(r) - O_{2,1+2}(r)] / \lambda_{1+2} = g_{1,1+2}(r) - g_{2,1+2}(r) \quad (1)$$

where $g_{1,1+2}(r)$ and $g_{2,1+2}(r)$ are the corresponding pair correlation functions (Wiegand and Moloney 2014). If DDM occurs, overall sapling density will be higher around dead saplings than around surviving ones, and consequently $DDM(r) > 0$. Conversely, if $DDM(r) < 0$, sapling survival will be higher in areas of high pre-mortality density and then we will encounter a pattern of density-dependent survival (DDS).

We generated 199 simulations of the random mortality null model and used the 5th lowest and highest values amongst these null patterns as simulation envelopes. In all analyses, we considered a maximum neighbourhood with a radius $r_{\max} = 50$ m. We selected this cut-off because it is larger than the spatial scale at which density-dependent effects among saplings are expected to drop off in the BCI plot; 30 m (Hubbell et al. 2001, Uriarte et al. 2005). To assess the overall fit of $DDM(r)$ under the random mortality null model over the 0-50 m distance range, we performed goodness-of-fit (GoF) tests (Loosmore and Ford 2006). When assessing the significance levels of the GoF tests, we applied Rice's sequential correction (Rice 1989) in order to avoid type I errors.

As a measure of the strength of conspecific DDM, we calculated the *z-scores* or *standardized effect sizes*; $DDM^{ses}(r)$ of the summary statistic $DDM(r)$ under the random mortality null model, by the following transformation:

$$DDM^{ses}(r) = \frac{DDM_{obs}(r) - \mathbf{M}[DDM_i(r)]}{\mathbf{SD}[DDM_i(r)]} \quad (2)$$

where $DDM_{obs}(r)$ is the summary statistic of the observed data, $DDM_i(r)$ is the summary statistic of the i th simulation of random mortality, and $\mathbf{M}[DDM_i(r)]$ and $\mathbf{SD}[DDM_i(r)]$ are the mean and the standard deviation of the values of $DDM_i(r)$ taken over $i = 1, \dots, 199$, respectively. For a particular distance r , if $DDM^{ses}(r) > 1.96$, we find density-dependent mortality and if $DDM^{ses}(r) < -1.96$ we encounter density dependent survival, with $P < 0.05$. The higher the absolute value of $DDM^{ses}(r)$, the stronger the departure from the random mortality null model. The effect sizes $DDM^{ses}(r)$ weigh up the observed departure from the expectation of random mortality (the numerator of eq. 2) with the uncertainty due to low sample sizes (the denominator of eq. 2). Therefore, they allow for comparisons between total DDM at community and light-guild levels. We used the maximum value of the effect sizes $DDM^{ses}(r)$ over the distance interval 1- 50 m as an indicator of the strength of conspecific and total DDM. Note, however, that the strength of conspecific DDM measured herein as the maximum value of the standardized effect sizes is different to what is

considered the strength of conspecific DDM in studies using individual-neighbourhood analyses, which is given by the regression coefficient of the models and expresses the degree to which the probability of survival of an individual is reduced by the addition of one conspecific neighbour (e.g. Comita et al. 2010).

To assess if the strength of conspecific DDM varied negatively with species abundance, in each period we performed a linear regression in which the effect size at representative neighbourhoods of $r = 5, 15$ and 30 m was the dependent variable and the total number of saplings was the independent variable.

Conspecific density-dependent mortality at community and light-guild levels (H5)

To assess the effects of DDM among conspecific saplings at community and light-guild levels we considered all saplings summed over species and all light-demanding and shade-tolerant saplings as potential focal individuals, respectively, as in the analyses of total DDM at the community and light-guild levels. In this case, however, we used only conspecific neighbours to estimate the neighbourhood density around focal individuals and we maintained the species-specific mortality rates in the null model (Wiegand and Moloney 2014). The technical implementation of this analysis is better explained in the Appendix A.

All point pattern analyses were performed with the software *Programita* (www.programita.org). We used the global (WM) edge correction method to deal with the edge effects caused by the points that are closer to the edge of the plot than the others (Wiegand and Moloney 2014).

Results

Conspecific density-dependent mortality at the species level (H1)

Only a small proportion of species (17.8%) showed evidence of conspecific density-dependent mortality (DDM) in at least one inter-census period (Table S1). For individual inter-census periods we detected spatial patterns indicative of conspecific DDM in less than 6.3% of the species (Table 1). Moreover, the distributions of the effect sizes of conspecific DDM in each period were centred around zero (i.e. values between -1.96 and 1.96), and only a small proportion of effects reached large values (i.e. $|DDM_{ses}(r)| > 4$) indicative of strong density-dependent effects (Figs. S1 and S2). This implies that conspecific DDM among saplings is not widespread and thus, that H1 was not confirmed. We also detected spatial patterns indicative of conspecific density-dependent survival, but only in less than 3.0 % of the species (Table 1; Fig S1).

Conspecific DDM and species abundance (H2)

Conspecific DDM occurred mostly in a small proportion (3.05 to 6.34%) of the species, and peaked in the second and fourth inter-census periods. However, the linear regressions mainly showed non-significant (either negative or positive) relationships between the effect sizes and the number of individuals of the species showing significant DDM, in all inter-census periods and in the entire 27-year period considered (Table S2).

Total DDM at the community level (H3)

The test statistic $DDM(r)$ revealed a significant total DDM in all inter-census periods. We also found that the range of distances and the strength of this process changed markedly over time (Fig. 1a), confirming hypothesis 3. Whereas the first inter-census period (1982-1985) did not show a significant signature of DDM (but rather of DDS, at large spatial scales), the second inter-census period showed an extraordinarily strong signature of total DDM over the entire 1-50 m distance range studied. In the third and fourth inter-census periods the spatial signature of total DDM was damped down, being marginally significant between 1995 and 2000. In the last two censuses, once again, we observed a clear spatial signature of total DDM, but this was apparent over a shorter range of distances (< 20 m) in relation to the first census interval. Over the 1982-2010 period, total DDM at community level showed a marked spatial signature over the entire range of distances (Fig. S3a).

Total DDM at the light-guild level (H4)

The test of density-dependent mortality among saplings of light-demanding species showed strong and highly significant positive deviations from the random mortality null model in all inter-census periods (Fig. 1b), which indicates total DDM. The spatial signature of this process showed little variation among census periods, but over the third and sixth ones, it became apparent over a longer range of distances (up to 30-40 m) than in the others. Spatial patterns indicative of total DDM among light-demanding species were also strongly marked up to distances of 25 m over the 1982-2010 period (Fig. S3b). We also found spatial patterns indicative of total DDM among saplings of shade-tolerant species, but they varied greatly over time (Fig. 1c). During the first three inter-census periods (up to 1995), these patterns were apparent at intermediate to large spatial neighbourhoods. However, they appeared over all distances analyzed from 1995 to 2000 and almost disappeared from 2000 onwards. Our hypothesis 4 is thus confirmed. Note, however, that total DDM among shade-tolerant saplings revealed a clear spatial signature over the entire 27-year period considered (Fig. S3c).

Conspecific DDM at community and light-guild levels (H5)

Our analysis of conspecific DDM at the community and light-guild levels detected only weak and marginally significant departures from random mortality over the six inter-census periods. The overall test statistic showed a marginally significant spatial signature of DDM at intermediate to large distances (> 15 m) over the second and third inter-census periods, and at intermediate neighbourhoods (5 – 25 m) in the sixth one (Fig. 2a). When we analysed only saplings of light-demanding species, this marginally significant spatial signature also became apparent at small scales (1 – 10 m) over the second, third, fifth and sixth inter-census periods (Fig. 2b). It was also detected at large scales (> 15 m) in the fourth and sixth inter-census periods when pooling all shade-tolerant species (Fig. 2c). We also detected a significant spatial signature of DDS in the first and fifth inter-census periods when pooling all species (Fig. 2a) and in the first period on combining the shade-tolerant ones (Fig. 2c). Comparison of figures 1 and 2 shows, in agreement with H5, that total DDM is stronger than conspecific DDM at the community and light-guild levels. This was confirmed by the maximum values of the effect sizes for the different cases (Table S3). However, significant departures from random mortality appeared when considering conspecific DDM at the community and light-guild levels over the entire 27-period, particularly for shade-tolerant species (Fig. S4) but they were weaker than their species-blind counterparts (Fig. S3, Table S3).

Discussion

Our detailed analyses of temporal changes in the occurrence and strength of density-dependent mortality (DDM) in the BCI forest indicate that conspecific DDM among saplings was not widespread and had no greater effect on saplings of the rare species than upon saplings of the most abundant ones. The spatial signature of conspecific DDM at the community- and light-guild levels was also generally weak. However, our results indicate strong effects of total DDM among all saplings and among those of light-demanding species. We also found pronounced temporal variability in the spatial signature of DDM among the different inter-census periods, over the 27 years studied. Our results confirm the trends found in some previous studies of DDM among saplings, but are in strong contrast with those for seedlings, which found strong negative effects of conspecific neighbours on the survival of focal individuals.

Conspecific density-dependent mortality

Contrary to our hypothesis 1, we found that conspecific DDM occurred only in a small proportion of species ($< 6.3\%$), with variable proportions depending on the inter-census period. Moreover, spatial point patterns indicative of this mechanism were consistent over two or more inter-census periods in only 6 species; *Anaxagorea panamensis*, *Chamguava schippii*, *Coussarea curvigemma*, *Faramea occidentalis*, *Mouriri myrtilloides* and *Tachigali versicolor*. Our

results are in contrast with those previously found by Peters (2003) in the same forest for the 1982-1995 data, indicating that conspecific NDD among saplings (all woody individuals with a 1-5 cm DBH) affected 51% of the species. These discrepancies can be explained by the fact that Peters (2003) used an individual-based approach to determine the influence of sapling density on tree survival in neighbourhoods with particular radii of 5, 10, 15, and 20 m from the focal tree. In contrast, we have considered a stricter criterion to consider conspecific DDM as significant (i.e. if departures from the random mortality null model at any distance were significant over the 0-50 m distance range).

Note also that, according to our findings, the proportion of species showing conspecific DDM among saplings in at least one inter-census period (17.8%) was much lower than that of seedlings (49% *sensu* Comita and Hubbell 2009). Similarly, Comita and Hubbell (2009) found a lower incidence of conspecific DDM among seedlings compared to that existing at the seed-to-seedling transition in the BCI forest (e.g. Harms 2000). They explained this result by the fact that the strong conspecific DDM during the seed-to-seedling transition could have thinned the local densities of some species below the levels at which conspecific DDM among seedlings was detectable. A similar explanation can apply for the lower proportion of species showing conspecific density-dependent mortality among saplings than among seedlings.

Species-specific habitat preferences may also obscure the spatial signature of conspecific DDM among saplings for many species, as has recently been indicated in a subtropical (Gutianshan; Zhu et al. 2010) and in a temperate (Liangshui; Piao et al. 2013) forest of China. These two forests, however, occur over steep, rugged terrain, whereas BCI presents a relatively gentle topography. On the other hand, mortality patterns resulting from habitat effects can be confounded with those originated by DDM when saplings located in abiotically stressful or infertile habitats die in clumps (Wiegand and Moloney 2014). But this process usually enhances the spatial signature of conspecific DDM (Bagchi et al. 2011), and in our case, this was not very evident. All this evidence suggests that the weak spatial signature of conspecific DDM that we found is not due to the confounding effects of species-specific habitat preferences.

The weak and marginally significant signature of conspecific DDM at community- and light-guild levels (Fig. 2, Fig. S4), compared to that of total DDM (Fig. 1, Fig. S3), is consistent with the small number of species exhibiting spatial patterns indicative of conspecific DDM in all inter-census periods and over the entire 27-year study period. This result can be explained by the cancelation of the effects of species showing density-dependent mortality and survival (Pueyo et al. 2007). It might be also related to the fact that only a few of the most abundant species showed conspecific DDM and that in turn, they did not affect the spatial signature of this process obtained when all species were pooled together.

In contrast with our hypothesis 2, we did not detect a significant negative relationship among the effect sizes and the number of individuals of the species. Note that in equation 2 the standard deviation of $DDM_{\lambda}(r)$ is proportional to

the number of individuals (see also p. 234 in Illian et al. 2008). This fact indicates that effect sizes will decline with n (i.e. the fewer individuals, the wider the simulation envelopes), which in turn suggests that the least abundant species should be subjected to stronger DDM than the most abundant ones. This difference in the strength of DDM, however, is not significant.

It is also important to point out that the mortality of focal saplings does not only depend on the density of neighbours at specific spatial scales, but also on the proximity of adult trees (Comita et al. 2014). In BCI, growth of focal saplings was affected by adult trees belonging to the same light-guild (Uriarte et al. 2005), and we suspect that sapling survival might be negatively impacted by conspecific large trees. These effects are beyond the scope of the present study, but they will be considered in forthcoming research.

Total density-dependent mortality

As predicted by hypotheses 3 and 4, total DDM (i.e. the effect of conspecific and heterospecific neighbours on a focal sapling) was strong for all saplings together and for those of light-demanding species. These results corroborate those obtained by Hubbell et al. (2001) and Uriarte et al. (2005) in the BCI plot up to 1995, confirming that they also apply over the 1995-2010 period. Thus, self-thinning and sheer crowding appear to be the most important drivers of sapling survival in this forest, regardless of the identity of neighbours. Given that saplings will compete strongly for space when they grow to larger sizes, we hypothesize that these mechanisms among saplings should also be detectable in other forests. Such patterns of total DDM can arise as a consequence of the prolonged effects of low levels of available light in the understory (Rüger et al. 2009), but they can also be caused by the attack of non-specific natural enemies (Luo et al. 2012). The spatial signature of total DDM at community level also exhibited great variability over the different census periods (Fig. 1a). In particular, it showed a noteworthy increase from 1982 to 1990 and a progressive decrease up to 2010. These findings can be explained by the large number of saplings recruited in 1985 as a consequence of the high tree mortality caused by the 1983 drought (Condit et al. 2004, Feeley et al. 2011). The saplings might have experienced strong DDM from 1985 to 1990, but from 1990 onwards, as soon as their distribution became progressively thinned, the strength of this mechanism might have diminished. The hypothesis of progressive self-thinning of the saplings recruited in the 1985 census up to 2010 is supported by the smaller number of total saplings in the community over the last three inter-census periods, in comparison with the first two (Table 1).

The mortality pattern of light-demanding saplings was strongly impacted by the density of all their light-demanding neighbours (Fig. 1b). This finding indicates that the predictions of Hubbell et al. (2001) for the same plot over the 1982-1985 period, according to which total density (i.e. density of conspecific and heterospecific individuals) was the first strongest factor negatively affecting survival of all free-standing woody plants ≥ 1 cm DBH, could be extended up to 2010. Our findings do not enable us to identify the ultimate cause of mortality of light-demanding

saplings, but we hypothesize that it is caused mainly by plant-plant competition for light. In the BCI forest, the saplings of light-demanding species appear almost exclusively in tree-fall gaps, where they form dense clumps (Hubbell et al. 1999, Schnitzer and Carson 2001). The greater light availability of tree-fall gaps increases the recruitment of new saplings, as well as the growth of those already present in the understory (Rüger et al. 2009), which in turn leads to asymmetric competition for light between larger and smaller neighbours (Stoll and Newbery 2005). The mortality of these saplings can also be augmented by the closing of gaps as the surrounding canopy of large trees progressively grows, rendering an increasingly unsuitable light climate (Wright 2003). Our hypothesis that total density-dependent mortality among light-demanding saplings was caused by competition for light seems plausible because the spatial signature of this process was apparent up to spatial scales of 25-35 m in many inter-census periods, and these scales match the < 50 m size of 83% of the tree-fall gaps in the BCI plot (Lobo and Dalling 2014). It is also supported by the finding that the range of spatial neighbourhoods in which total DDM among light-demanding saplings occurred was wider over the 1990-1995 and 2005-2010 inter-census periods. In the BCI forest, two unusually severe droughts associated with El Niño events occurred in 1983 and 1998. These droughts, particularly the first one, provoked high tree mortalities and subsequent canopy openings (Condit et al. 2004), which increased light availability and in turn, sapling growth and mortality through self-thinning (Schnitzer and Carson 2001). Nonetheless, density-dependent effects among saplings may take years or even decades to become evident (Zhu et al. 2010), which might explain why total DDM among light-demanding saplings was apparent 10-15 after the occurrence of canopy openings in 1983 and 1998 (i.e. over the 1990-1995 and 2005-2010 inter-census periods).

In agreement with hypothesis 4 we found that total DDM among saplings of shade-tolerant species was much weaker than that of light-demanding species. Moreover, the spatial signature of this process was apparent at intermediate to large distances from focal plants (i.e. from $r = 5-10$ m onwards). This type of spatial pattern suggests the existence of large, high-density patches of dead saplings of shade-tolerant species in the understorey of this forest (Raventós et al. 2010). These patches may be caused by the attack of non-specific natural enemies, in particular by *polyphagous* pathogens (i.e. pathogens with several species of host plants, Webb et al. 2006), or by wood decaying fungi, which propagate rapidly, affecting a large proportion of live trees in BCI (Gilbert 2005). Shade-tolerant saplings might have also died as a consequence of the effects of low light availability over time (Rüger et al. 2009). It is also important to note that, although total DDM was much weaker among the shade-tolerant saplings than among the light-demanding ones, the spatial signature of conspecific DDM at the light-guild level was clearly marked for shade-tolerant species over the entire 27-year period (Fig. S4c). This result could be explained by the fact that the number of recruiting saplings of shade-tolerant species was much lower than that of the light-demanding ones in each of six inter-census periods and therefore, the cumulative effects of an increasing proportion of dead vs. surviving shade-tolerant saplings clearly appeared when considering the entire 27-year period.

Conclusions

Our study of density-dependent mortality (DDM) among saplings in the moist tropical forest of Barro Colorado Island, over six inter-census periods (27-yr), reveals two main novel insights. First, it indicates that, among saplings, conspecific DDM (i.e. the density-dependent mechanism of the *Janzen-Connell hypothesis*) is relatively unimportant compared to total DDM (i.e. the effect of the overall neighborhood stem density on a focal sapling) at community and light-guild levels. This finding is in strong contrast with those obtained by studies on DDM among seedlings (Comita and Hubbell 2009, Comita et al. 2010, 2014, Lebrija-Trejos et al. 2014), and points toward the occurrence of a fundamental shift in the operation of density-dependent effects over the early stages of tree development; at the seedling stage density-dependent mortality occurs mainly among conspecific individuals, but becomes *species-blind* when occurring among saplings, which are less abundant in the understory. This shift involves important ecological implications. At the seedling-to-sapling transition, DDM occurs among conspecific individuals and is of vital importance with regard to fostering species coexistence in tropical forests (Wright 2002). However, at the sapling-to-adult transition, density-dependent mortality is largely random in relation to species, which suggests that its occurrence is not so critical in the regulation of species diversity through the density-dependent mechanism of the *Janzen-Connell hypothesis* (Janzen 1970, Connell 1971). It could play a role, however, through the mechanisms pertaining to the *regeneration niche hypothesis* (i.e. temporal and spatial variations in environmental conditions enable species to coexist; Denslow 1987).

Second, our results support the idea that total density dependent mortality among saplings is an important driver of the structure and dynamics of moist tropical forests (Hubbell et al. 2001, Uriarte et al. 2005). However, the strong temporal variations in the spatial signature and strength of this process indicate that it is strongly affected by time-varying events such as tree-fall gaps and episodes of high recruit emergence. These events enhance sapling growth and mortality, cause sharp increases in the abundance of light-demanding saplings and might be associated with the severe droughts that occurred after El Niño events, particularly in 1983 (Condit et al. 2004), but they could also be reflecting increased regional aridity resulting from long-term climate change (Feeley et al. 2011). If this is the case, we predict that the moist tropical forests of the Panama isthmus, such as the one in BCI, would undergo an acceleration of gap-phase regeneration processes (Schnitzer and Carson 2001, Lobo and Dalling 2014), with sharp emergence of new recruits, a higher growth rate of the existing saplings, and the subsequent mortality of the smaller neighbours through total density dependent effects. These mechanisms would also increase the importance of niche differentiation processes at small scale (< 5 m from the focal plant; Velázquez et al. *in press*), and might be particularly marked if we consider that the frequency and intensity of El Niño events are predicted to be greater during the coming decades (Vandermeer et al. 2001).

It should be noted that we obtained these findings by assessing density-dependent mortality in a permanent forest plot (BCI) providing an exceptionally long-term dataset, which highlights the need for longer time intervals in order to study how climate shifts affect forest structure and function (Clark 2007, Feeley et al. 2011). Similar analyses should be conducted in other permanent plots as soon as data from new censuses become available, to test whether these trends also apply to other forests.

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Table Legends

Table 1. Characteristics of the sapling community in the moist tropical forest of Barro Colorado Island (Panama), over the six inter-census periods considered. (i) Total number of species and number of species with > 70 saplings, (ii) number of saplings of all species, light-demanding and shade-tolerant species, and species with > 70 saplings, (iii) percentage of dead saplings in all, light-demanding and shade-tolerant species, and (iv) percentage of species showing spatial patterns indicative of density-dependent mortality (DDM), density-dependent survival (DDS) and no effect. Note that the numbers of saplings of light-demanding and shade-tolerant species do not add up to the number of species with > 70 saplings because there were also a large number of intermediate and non-determined species (Comita et al. 2010).

Inter-census period	1982-1985	1985-1990	1990-1995	1995-2000	2000-2005	2005-2010	1982-2010
Number of species							
Total	283	285	279	275	273	270	285
> 70 saplings	131	126	123	113	112	113	131
Number of saplings							
All species	181159	185920	179132	164721	150911	146344	181159
Light-demanding species	4811	4741	5340	4253	3939	4338	4725
Shade-tolerant species	90300	93059	90782	86646	80562	77845	91029
Species > 70 saplings	169158	170630	170164	158293	144641	139645	169954
Dead saplings (%)							
All species	8.48	11.44	11.90	13.49	11.40	11.42	70.91
Light-demanding species	20.24	28.64	30.97	33.43	30.46	36.16	68.79
Shade-tolerant species	6.56	8.49	8.27	10.36	9.14	8.81	64.94
Species showing significant DDM (%)							
Species showing significant DDM (%)	3.05	6.34	4.87	5.30	4.46	5.30	8.94
Species showing significant DDS (%)							
Species showing significant DDS (%)	3.05	1.58	1.62	2.65	2.67	1.76	4.87
Species showing no effect (%)							
Species showing no effect (%)	93.9	92.08	93.51	92.05	92.87	92.94	86.17

Figure Legends

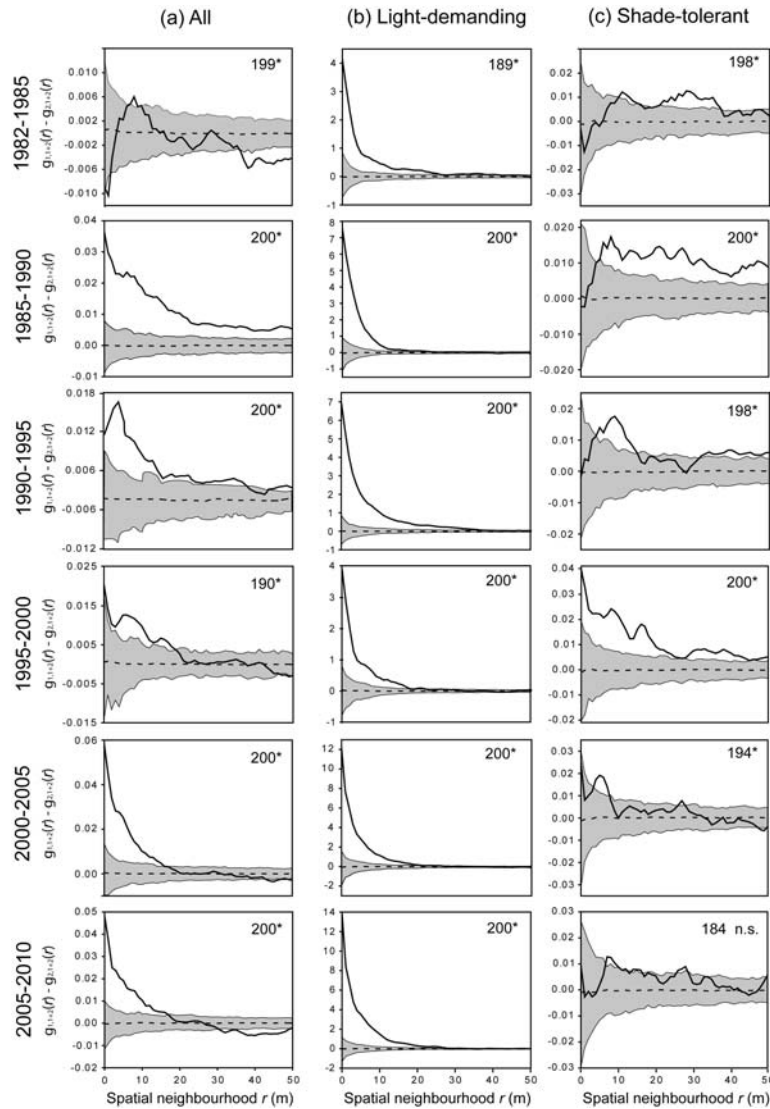


Figure 1. Analysis of total density-dependent mortality among (a) all saplings, and (b) saplings of light-demanding and (c) shade-tolerant species, in the Barro Colorado Island Forest Dynamics Plot, Panama, over the six inter-census periods. To detect density-dependent mortality we used the summary statistic $DDM(r) = g_{1,1+1}(r) - g_{2,1+2}(r)$ that compares the mean density of saplings at distance r of dead saplings (subscript 1) with that around surviving saplings (subscript 2). The observed $DDM(r)$ (bold lines) was contrasted with simulation envelopes (grey area) of the random mortality null model, being the 5th highest and lowest values of $DDM(r)$ taken from 199 simulations of the null model. If the observed $DDM(r)$ wanders above the simulation envelopes DDM exists and if it wanders below DDS occurs. On each panel, the ranks of the Goodness-of-Fit tests (GoF) are also shown together with their significance levels over the entire range of spatial neighbourhoods considered; ** $P < 0.001$, * $P < 0.05$.

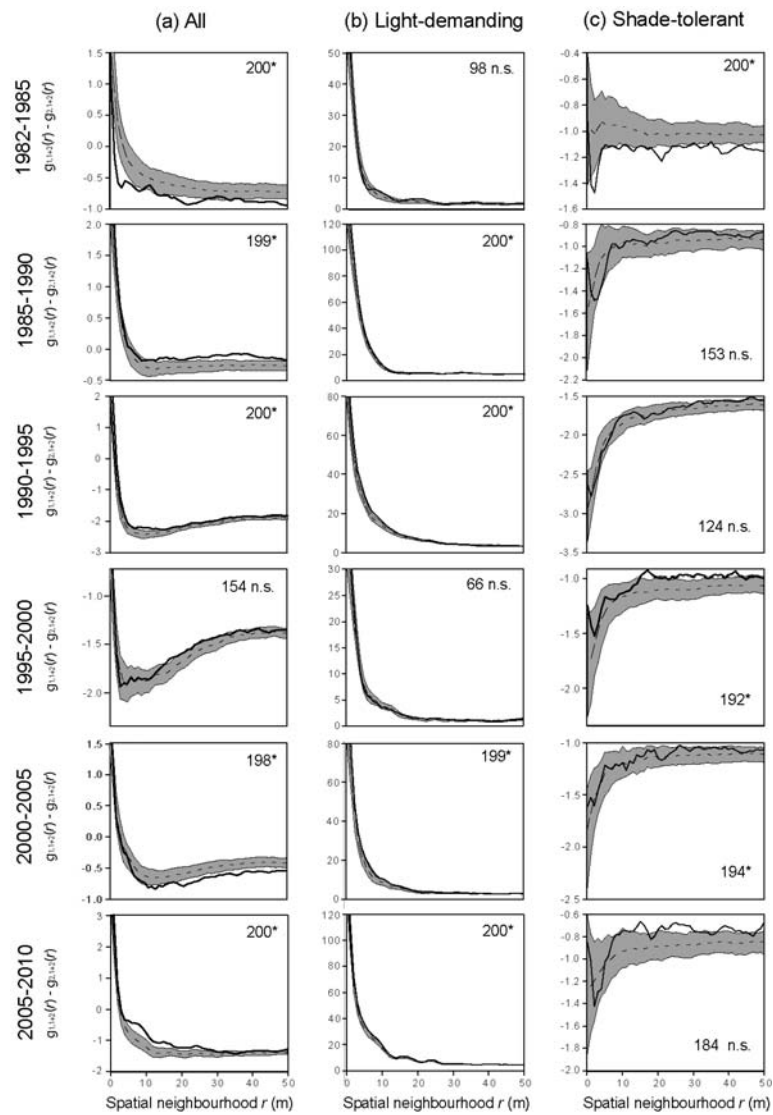


Figure 2. Analysis of conspecific density-dependent mortality among saplings for (a) all species (i.e. community level), (b) light-demanding and (c) shade-tolerant species (i.e. light-guild level), in the Barro Colorado Island Forest Dynamics Plot, Panama, over the six inter-census periods. Other conventions as in Figure 1.