Phylogenetic density dependence and environmental filtering predict seedling mortality in a tropical forest

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Abstract

Negative density dependence (NDD) and environmental filtering (EF) shape community assembly, but their relative importance is poorly understood. Recent studies have shown that seedling's mortality risk is positively related to the phylogenetic relatedness of neighbours. However, natural enemies, whose depredations often cause NDD, respond to functional traits of hosts rather than phylogenetic relatedness *per se*. To understand the roles of NDD and EF in community assembly, we assessed the effects on seedling mortality of functional similarity, phylogenetic relatedness and stem density of neighbouring seedlings and adults in a species-rich tropical forest. Mortality risks increased for common species when their functional traits departed substantially from the neighbourhood mean, and for all species when surrounded by close relatives. This indicates that NDD affects community assembly more broadly than does EF, and leads to the tentative conclusion that natural enemies respond to phylogenetically correlated traits. Our results affirm the prominence of NDD in structuring species-rich communities.

Keywords

Community assembly, density dependence, French Guiana, generalised linear mixed models, Janzen–Connell hypothesis, seedling recruitment, species coexistence, survival.

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INTRODUCTION

An inverse relationship between local conspecific density and individual performance is expected to promote species coexistence (Chesson 2000). The extraordinary species richness of many tropical rain forests has spurred numerous investigations for evidence of this relationship, referred to as negative density dependence (NDD). Following the seminal community-level test of NDD by Harms *et al.* (2000), a rush of studies have shown this process to be pervasive, particularly during seedling recruitment (Comita *et al.* 2010; Gonzalez *et al.* 2010; Metz *et al.* 2010). Nevertheless, the contribution of NDD to community assembly, relative to other processes such as environmental filtering (EF), remains a subject of debate.

Trophic interactions, either via resource competition or via natural enemies, are generally understood to generate NDD (Janzen 1970; Connell 1971). In the understory of tropical forests, tree seedlings are generally too scarce and too small to compete substantially among themselves for resources (Paine *et al.* 2008; Svenning *et al.* 2008), so NDD is unlikely to result from direct seedling–seedling competition. Adult-seedling competition, in contrast, is intense and asymmetric, strongly reducing seedling growth and survival (Lewis & Tanner 2000). The detrimental effects of adults on seedlings do not depend on the stem density of the seedlings, however, so NDD is unlikely to

result from adult-seedling competition. Rather, NDD is more likely to be generated by the depredations of natural enemies, by which we refer broadly to the insects, fungi and pathogens that consume or kill plants (Bagchi *et al.* 2010; Swamy & Terborgh 2010; Alvarez-Loayza & Terborgh 2011).

Recent studies have identified the phylogenetic relatedness of neighbours to be an important predictor of NDD, a pattern referred to as phylogenetic density dependence (Webb *et al.* 2006; Gonzalez *et al.* 2010; Metz *et al.* 2010; Ness *et al.* 2011). This pattern is consistent with observations that herbivorous insects are frequently associated with clades of host plants (Novotny *et al.* 2010), and that pathogen transmission between pairs of tree species is more likely if the species are phylogenetically related (Gilbert & Webb 2007). Applications of the phylogenetic approach are also limited, however, because the mechanisms causing NDD are likely to depend on plant functional traits, rather than species phylogenetic relatedness *per se*. In particular, natural enemies respond to physical and chemical properties of the plant tissues they attack (Coley & Barone 1996). Thus, evidence of phylogenetic density dependence only indicates the degree to which phylogenetically correlated plant traits serve as criteria for host-plant selection by natural enemies. The phylogenetic approach cannot detect the responses of natural enemies to phylogenetically labile functional traits. This prompts a reconsideration of the prevalence and

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strength of phylogenetic signal in functional traits (Losos 2008). The limited evidence on phylogenetic signal among traits that may influence host-plant selection shows contrasting patterns (Donoghue 2008; Swenson & Enquist 2009). For example, patterns of chemical defence are conserved within some taxa, such as terpenes in Burseraceae (Becerra 1997; Fine *et al.* 2006), but highly labile in others, such as *Inga* (Fabaceae; Kursar *et al.* 2009). To obtain clearer insights, we propose that studies of NDD consider both the evolutionary history and functional traits of neighbouring plants.

Studying community assembly through the lens of functional traits allows comparisons of the effects of EF and NDD. EF, also known as species sorting, affects community composition when individuals with functional traits incompatible with their environment experience elevated rates of mortality (Harper 1977). Recent studies have shown EF to powerfully shape community assembly in tropical forests (Kraft *et al.* 2008; Paine *et al.* 2011). To evaluate the effects of NDD and EF, we make four predictions about patterns of seedling mortality with regard to functional dissimilarity, phylogenetic relatedness, and stem density of neighbouring plants (Table 1). (1a) If natural enemies select host plants on the basis of functional traits, seedlings surrounded by other seedlings with similar traits would suffer increased mortality rates. Similarly, seedlings growing near functionally similar adults would have greater mortality rates, since adults can serve as reservoirs for populations of natural enemies (Janzen 1972; Metz *et al.* 2010). (1b) If, on the other hand, EF is strong, seedlings with trait values different from the neighbourhood mean would suffer elevated rates of mortality. We do not predict substantial EF based on seedling and adult functional traits because, from a seedling's perspective, adults in closed-canopy forests impose intensely asymmetric resource competition regardless of functional dissimilarity owing to their much greater stature (Lewis & Tanner 2000). (2) If the traits that natural enemies use for host selection are phylogenetically correlated, then increasing the phylogenetic relatedness of neighbours (or equivalently, increasing the similarity of those traits) would increase mortality rates. (3) Finally, if natural enemies operate in a strictly host-specific manner, then increasing conspecific or total stem density would increase mortality rates, whereas phylogenetic or functional similarity would have no effect.

In this study, we assessed the support for these four predictions in a diverse tropical tree community in French Guiana by synthesising extensive seedling and adult census data with an unprecedented

functional trait database and a species-level molecular phylogeny. We examined the effects of neighbouring seedlings and adults separately to determine the degree to which their effects were complementary. In addition to analysing the mortality patterns in the seedling layer as a whole, we analysed subsets representing relatively common and relatively rare species to determine the degree to which the support for each prediction pervades the community. The support for each prediction leads to differing inferences regarding relative importance of NDD and EF for community assembly, and are suggestive of mechanisms that generate NDD in high-diversity forests.

MATERIAL AND METHODS

Study site

This study was conducted in pristine tropical rainforest at the Nouragues Biological Station in central French Guiana (4°05′ N, 52°40′ W; Bongers *et al.* 2001). Annual rainfall averages 2990 mm with a dry season from September to November. The study area consists of two plateaus dominated by high mature forest in which a grid network of permanent forest plots covering *c.* 85 ha was established. One of the plateaus, Grand Plateau, is on clay soil with a metamorphosed volcanic substrate, and the other, Petit Plateau, is on sandy granitic-derived soil.

Data acquisition

We assembled four datasets to conduct this study: censuses of seedlings and adult trees, functional traits, and a community phylogeny.

Seedlings were censused in the context of the Nouragues Plant Regeneration Monitoring System at seven times between February 2004 and February 2009, with two censuses in 2004 and 2005, and single censuses in 2006, 2007 and 2009 (Norden *et al.* 2007). Stations, each with two or three 1-m^2 plots, were arranged every 15–50 m on alternate sides of trails, for a total of 95 stations, 201 plots, 3471 seedlings and 178 species. At each census, all woody seedlings with initial height between 5 and 100 cm were mapped, tagged, and identified to species or morphospecies. In the final census, un-identified seedlings were photographed to help synonymise morpho-

Table 1 The selection of host plants by natural enemies is likely to underlie the widely observed pattern of negative density dependence in seedling mortality. This table summarises the patterns in seedling mortality to be expected depending upon the criteria that natural enemies use for host-plant selection

	Prediction	
Mechanism	Seedling neighbourhood	Adult neighbourhood
(1a) Natural enemies select host plants on the basis of functional traits.	Increasing functional dissimilarity would decrease mortality.	If functionally similar adults are reservoirs for natural enemies, increasing dissimilarity would reduce mortality.
(1b) Environmental filtering eliminates seedlings with traits that are locally incongruous.	Increasing functional dissimilarity would increase mortality.	Little effect expected, since adults impose substantial asymmetric competition on seedlings, regardless of functional dissimilarity.
(2) Natural enemies use phylogenetically correlated functional traits.	Increasing phylogenetic relatedness (or, equivalently) increasing trait similarity would increase mortality.	If natural enemies utilise adults as reservoirs, increasing phylogenetic relatedness or increasing trait similarity would increase mortality.
(3) Natural enemies are strictly host specific.	Increasing conspecific or total stem density would increase mortality.	If natural enemies are prevalent and utilise adults as reservoirs, increasing conspecific or total stem density would increase mortality.

species among plots. The height of each seedling was carefully measured upon recruitment into the study; the imprecision of subsequent height measurements precluded analysis of height growth. In all, 47% of individuals were identified to species or morphospecies, 57% to genus, 75% to family and 25% remained unidentified. The latter group was excluded from further analysis, as it was not possible to calculate their dissimilarity with neighbouring seedlings or adults.

In November 2009, all trees ≥ 10 cm DBH within 15 m of trails (total area: 6.6 ha) were mapped, measured and identified to one of 502 species. We refer to all 3626 mapped trees as adults, although information on their actual reproductive status was lacking. Experienced botanists identified as many trees as possible from the ground using bark and trunk characteristics. Subsequent teams of climbers collected leaf samples from all trees not identified to species for later identification in the Herbier de Guyane. In all, 80% of adults were identified to species, 89% to genus, 97.4% to family and 2.6% remained unidentified.

We sampled functional traits on 4672 individual trees (representing 644 species) in nine 1 ha plots of lowland rain forest in French Guiana between November 2007 and September 2009 (Baraloto *et al.* 2010a). Additional trait samples were obtained from 209 individuals and 131 species during the census of adult trees, for a total trait dataset of 4881 individuals and 719 species. Species means were used for trait values. We used two broad criteria to select functional traits for analysis: that the traits are relevant to the performance of both adults and seedlings, and that they are relatively easy to acquire. For the current study, we selected four functional traits that are correlated with the global leaf economics spectrum: foliar chlorophyll content, C:N ratio, toughness and specific leaf area (SLA; Wright *et al.* 2004; Baraloto *et al.* 2010b). These traits are linked to a resource acquisition-conservation tradeoff, but also define a gradient of palatability that is relevant to natural enemies (Coley & Barone 1996). The inclusion of traits that are even more strongly linked to plant fitness or anti-herbivore defence, such as quantum yield or defensive chemistry profiles (Kursar *et al.* 2009), would have been ideal, but was infeasible given the taxonomic scope of the study. Importantly, the functional traits we investigated show limited, though significant, phylogenetic signal in the forests of French Guiana (Chave, Dexter, Paine & Baraloto, *unpublished data*). This allowed us to independently investigate the degree to which natural enemies select host plants on the basis of both measured evolutionarily labile traits and unmeasured phylogenetically correlated traits (Table 1). Host-plant selection on the basis of unmeasured labile traits, contrastingly, would not be detectable.

We assembled a species-level community molecular phylogeny for 627 species, which were sequenced for the *mat*K and *rbc*L chloroplast genes. For details of extraction, PCR, and sequencing, see Gonzalez *et al.* (2009). See Appendix S1 in Supporting Information for details of the phylogenetic tree reconstruction.

Analyses

We predicted the mortality of individual seedlings based upon the functional dissimilarity, phylogenetic relatedness and stem density of their seedling and adult neighbours. Functional trait dissimilarity was calculated as the Euclidean distance among species in multivariate trait space. Trait values were standardised to a mean of zero and unit variance so that all traits are equally weighted in the calculation of functional dissimilarity. Functional dissimilarity, as a distance in scaled multivariate trait space, has no interpretable units, but larger values

indicate more dissimilar pairs of species. Phylogenetic relatedness was assessed as branch length (cophenetic) distance, equivalent to the APd' statistic of Webb *et al.* (2006), and has units of millions of years (Mya). For every seedling, we calculated the mean dissimilarity between it and its seedling and adult neighbours. Rather than arbitrarily selecting a neighbourhood size, we accounted for variation in the stature and proximity of neighbours by weighting pairwise similarities positively by the height of the neighbouring seedling or the basal area of the neighbouring adult, and inversely by the square of the distance between the focal seedling and its neighbour. Since seedling community dynamics are rapid (Norden *et al.* 2007), we calculated similarities for each seedling at each census. The dynamics of adult trees is slower, so we assumed seedling-adult dissimilarity to be static over the 5 years in which we observed seedling mortality. Because not all species were present in each of the four datasets (seedlings, adults, traits and phylogeny), we substituted means of the functional and phylogenetic similarities for the next higher taxonomic level for taxa that were not present in the relevant datasets.

We predicted the mortality of each individual seedling through each census interval with generalised linear mixed models, using a Bernoulli response with a complementary log-log link implemented in the lmer4 package of R (R Core Development Team 2010). Because the census intervals were irregularly spaced in time, the log-transformed time since the previous census was used as an offset, which produces parameter estimates in units of years, rather than census intervals (Egli & Schmid 2001). To evaluate the possible complementarity in the effects of seedlings and adults on seedling mortality, we constructed models based on the seedling neighbourhood alone, the adult neighbourhood alone, and both neighbourhoods together. All models included the initial size of focal individuals, since, all else being equal, mortality rates decline with increasing seedling stature. Smaller seedlings are more susceptible to a wide range of mortality agents, such as falling branches, shading, and herbivory (Leishman *et al.* 2000). Pairwise interactions between seedling height and the similarity metrics were included in the models since the effects of neighbours may accumulate or diminish with increasing seedling size. Seedling neighbourhood models included a term for seedling plot-level stem density, since dense crowds of seedlings may increase competition among seedlings or facilitate the transmission of natural enemies (Janzen 1970; Connell 1971; Webb *et al.* 2006). To allow comparisons among parameter estimates, all predictor variables were centred and scaled by their standard deviation. Thus, a one-unit change in the value of a predictor corresponds to a change of one standard deviation. The effect of conspecific density was assessed only for the 20 most-common species, as rarer species almost always occurred as singletons, meaning that the effects of conspecific density could not be evaluated. Finally, to determine the degree to which the detected patterns were general across the community, we excluded the 20 most-common species.

We used a stepwise variable selection procedure based on the Bayesian Information Criterion (BIC), to pare away non-informative fixed effects. We used BIC, instead of the more common AIC, since it is more parsimonious for large datasets (Burnham & Anderson 2002). Beginning with the most-complicated model, we built models in which each fixed effect was eliminated in turn. Each of these simplified models was compared with the more-complicated model on the basis of BIC. The process was repeated until the most parsimonious model was determined. All models included the random effects of seedling plot, seedling cohort (the census at which each

seedling was recruited into the study) and census date. This focused the analysis on NDD while accounting for geographic and temporal variation in mortality rates, which can be substantial (Norden *et al.* 2007). We also tested models with more complicated random effects, including allowing the effects on seedling mortality of height and neighbourhood similarity to vary among plots and census dates. Intercept-only random-effects structures were preferred by BIC for the seedling and seedling-adult models, whereas allowing the effects of height on mortality to vary among plots was preferred in the adult-only model. Functional dissimilarity, stem density and seedling height were log-transformed prior to analysis to improve normality, and back-transformed for presentation.

RESULTS

We modelled seedling mortality as a function of the seedling neighbourhood, the adult neighbourhood, and both neighbourhoods together. The combined model was preferred on the basis of the Bayesian Information Criterion (BIC), indicating that the seedling and adult neighbourhoods had complementary effects on seedling mortality (Table 2). The estimated coefficients of the predictor variables were similar in value in the combined seedling-adult model and when considering the two neighbourhoods separately, indicating that the effects of neighbouring seedlings and adults on mortality rates were additive, rather than multiplicative.

Seedlings surrounded by functionally dissimilar and closely related seedlings had elevated mortality rates (Figs 1a and b), whereas total

Table 2 Summaries of three generalised mixed models predicting seedling mortality on the basis of seedling and adult functional dissimilarity and phylogenetic relatedness. Predictor variables were centred and standardised, meaning that a one-unit change in each parameter (apart from the intercept) corresponds to a change of one standard deviation. Negative parameter estimates indicate parameters that reduce seedling mortality rate (year^{-1}). All parameters are significant at the level of $\alpha = 0.05$. Models are ranked by ΔBIC

	Estimate	SE	z-value	P value
Seedling and adult neighbourhoods together, ΔBIC: 0				
(Intercept)	−1.719	0.201	−8.543	< 0.0001
Seedling height	−0.479	0.035	−13.647	< 0.0001
Seedling–seedling functional dissimilarity	0.186	0.035	5.248	< 0.0001
Seedling–seedling phylogenetic relatedness	−0.251	0.030	−8.402	< 0.0001
Seedling-adult phylogenetic relatedness	−0.082	0.027	−3.000	0.0027
Height × seedling–seedling functional dissimilarity	0.070	0.033	2.104	0.0354
Height × seedling–seedling phylogenetic relatedness	−0.062	0.029	−2.116	0.0343
Seedling neighbourhood only, ΔBIC: 11.1				
(Intercept)	−2.017	0.286	−7.056	< 0.0001
Seedling height	−0.480	0.035	−13.685	< 0.0001
Seedling–seedling functional dissimilarity	0.190	0.035	5.384	< 0.0001
Seedling–seedling phylogenetic relatedness	−0.263	0.029	−8.945	< 0.0001
Height × seedling–seedling functional dissimilarity	0.067	0.033	2.012	0.0442
Height × seedling–seedling phylogenetic relatedness	−0.061	0.029	−2.109	0.0349
Adult neighbourhood only, ΔBIC: 98.0				
(Intercept)	−2.001	0.287	−6.964	< 0.0001
Seedling height	−0.459	0.041	−11.120	< 0.0001
Seedling-adult phylogenetic relatedness	−0.114	0.027	−4.234	< 0.0001

stem density had no effect. Functional dissimilarity and phylogenetic relatedness both interacted with seedling height, such that their effects intensified with increasing stature. Phylogenetic relatedness had an approximately 30% stronger effect on per-capita mortality risk than did functional dissimilarity (mean ± SEM: −0.25 ± 0.03% and 0.19 ± 0.04%, respectively). In contrast to the strong effects of neighbouring seedlings, neighbouring adults affected seedling mortality in a relatively minor way. Increasing seedling-adult phylogenetic relatedness modestly increased the probability of mortality (−0.082 ± 0.027%; Fig. 1c). Conspecific density had no significant effect on the mortality of the 20 most-common species when considering those species jointly (Appendix S2). Considering each common species separately, conspecific density (and its interaction with seedling height) explained significant variation in mortality for five of 20 species, including three of the five most common species.

To better understand the relationship between functional dissimilarity and seedling mortality, we sought to identify which traits most strongly contributed to the patterns observed. We calculated similarities for each of the four functional traits, and substituted these univariate measures for multivariate functional dissimilarity in the joint seedling-adult neighbourhood model. Following the same procedure of model selection as described above, we obtained a model similar to the original, but with functional dissimilarity replaced by dissimilarity in SLA (Appendix S2). Thus the effect of functional dissimilarity in the original analysis appeared to be most strongly related to variation in SLA.

The inclusion of traits measured on individuals far from the study location may bias our results (Baraloto *et al.* 2010a). To assess this, we repeated our analyses, restricting the functional trait dataset to those 1260 trees measured for traits at the Nouragues Biological Station. These analyses yielded results qualitatively identical to the overall analysis, so we report only results from the complete trait database.

To assess the degree to which the observed patterns were general across the seedling layer, we excluded the 20 most-common species, which numerically dominated the overall analysis. Analysis of the rarer species yielded similar results to the overall analysis, with the important difference that functional dissimilarity explained no significant variance in their mortality (Appendix S2).

To weigh the implications of using a phylogenetic tree resolved to the level of species, we collapsed all family-level nodes in the original tree to polytomies, thereby creating a tree that lacked resolution within families and genera and approximated the topology of the APG III backbone phylogeny (APG III 2009). We recalculated phylogenetic relatedness using the modified tree and repeated the combined seedling mortality analyses. Results were broadly similar to those obtained from the species-resolution phylogeny: increasing phylogenetic relatedness increased seedling mortality, whereas increasing functional dissimilarity increased seedling mortality (Appendix S2).

To assess the potential for bias introduced by assigning trait values and phylogenetic relationships at levels above that of species, we repeated the analysis after excluding all seedlings not identified to the species level. Analysis of this positively identified subset of seedlings yielded a model qualitatively similar to the overall seedling-only model (Appendix S2). This indicates that imprecision in seedling identification, though it may have reduced the sensitivity of our analyses, it did not generate spurious relationships.
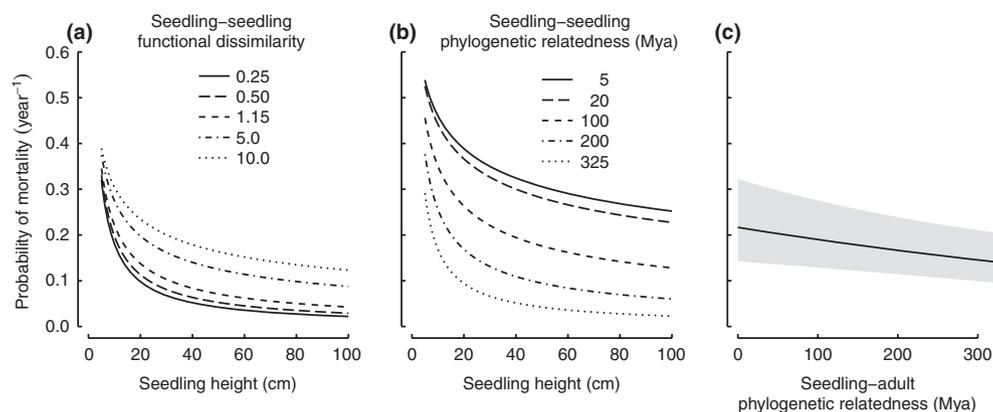
Figure 1 Seedling mortality as predicted by functional dissimilarity and phylogenetic relatedness with neighbouring seedlings and adults. (a) Seedling height and seedling functional dissimilarity interact such that larger seedlings suffer disproportionately high mortality rates when growing near neighbours with highly divergent functional traits. (b) Seedling height also interacts with seedling phylogenetic relatedness, such that larger seedlings suffer disproportionately high mortality in neighbourhoods of closely related seedlings. (c) Increasing phylogenetic relatedness among adults and neighbouring seedlings also reduced seedling mortality rates. Analyses were performed on centred and standardised predictors and were back-transformed for interpretability. Grey bands indicate expected mortality rate ± 1 SEM. Mya, Millions of years.

DISCUSSION

We tested the relationships between seedling performance – measured as mortality – and the functional dissimilarity, phylogenetic relatedness and stem density of neighbouring plants in a tropical forest. Seedling mortality increased in neighbourhoods of functionally dissimilar seedlings (Fig. 1a). Thus, there was no support for the hypothesis that the intensity of NDD increases with functional trait similarity. Rather, the interpretation is that EF on the measured traits was intense. Simultaneously, seedling mortality increased in neighbourhoods of closely related seedlings and adults (Fig. 1b and c), indicating that NDD is positively related to (unmeasured) phylogenetically correlated functional traits. The positive phylogenetic relatedness-mortality relationship is consistent with the positive dissimilarity-mortality relationship, because the functional traits assessed in this study showed little phylogenetic signal. The effect of phylogenetic relatedness was approximately 30% stronger than that of functional similarity, which was only detected among common species. Thus, natural enemies appeared to shape community assembly more strongly and more pervasively than did EF. Finally, conspecific and total stem density had little effect on seedling mortality (Table 2).

What mechanisms underlie the observed patterns of NDD? Possibilities include inter-seedling competition, adult-seedling competition, and predation by natural enemies. We discount the former two owing to the scarcity and small stature of seedlings in these forests, and the observation that the strength of asymmetric adult–seedling competition is largely independent of seedling density (Lewis & Tanner 2000; Paine *et al.* 2008). By process of elimination therefore we suggest that natural enemies were the primary generators of NDD in this study. In the following paragraphs, we discuss the implications of these results for seedling recruitment and the assembly of species-rich plant communities.

The effects of functional dissimilarity

Interpreting the effects of functional dissimilarity on seedling mortality is complicated by the fact that two countervailing mechanisms affect the observed pattern (Table 1). Here, because seedling mortality increased with increasing neighbourhood functional dissimilarity, we infer that EF was strong on the leaf economics spectrum (with which the four traits we measured were correlated; Wright *et al.* 2004; Baraloto *et al.* 2010b). This is consistent with previous findings that EF shapes community assembly in neotropical rain forests (Kraft *et al.* 2008; Paine *et al.* 2011). The importance attributed to EF depends upon the gradient of environmental conditions examined. Had we conducted this study across a wider range of environmental variation, rather than focused on closed-canopy *terra firme* rain forest, an even greater role of EF may have been detected (Fine *et al.* 2006; Paine *et al.* 2011). In contrast, had the depredations of natural enemies been positively correlated with functional similarity, the inverse relationship would have been observed (Table 1). Such a relationship is plausible, because chlorophyll content and C:N ratio are indicative of the nutritive benefit of leaves, and toughness and SLA are related to their palatability (Coley & Barone 1996). That we did not observe such a relationship indicates that either natural enemies do not select host plants on the basis of these particular functional traits, or if they do, that the effect is so weak as to be swamped by the countervailing effect of EF.

The inclusion of functional traits in neighbourhood analyses of seedling mortality is predicated on the assumption that trait values measured on adults may be applied to conspecific seedlings. In French Guiana, seedling-adult correlations of leaf traits are strong, though adults tend to have tougher leaves with lower SLA than do seedlings (Courtois 2010). Adult and seedling leaves also exhibit similar leaf carbon isotope discrimination and photosynthetic potential on an area basis (Thomas & Bazzaz 1999; Bonal *et al.* 2007). In the most complete study on the subject, 12 of 14 traits were strongly correlated between field-grown adults and laboratory-grown seedlings, both in terms of trait values and species ranks (Pearson's *r*: 0.59 ± 0.14, Spearman's ρ: 0.61 ± 0.12; Cornelissen *et al.* 2003). These studies indicate that for many functional traits, both trait values and species rankings tend to remain broadly consistent over ontogeny, justifying our application of adult trait values to conspecific seedlings.

The effects of phylogenetic relatedness

Confirming the results of previous studies, seedling mortality increased in neighbourhoods of individuals with similar evolutionary

histories (Webb *et al.* 2006; Gonzalez *et al.* 2010; Metz *et al.* 2010). This pattern is consistent with the behaviour of natural enemies, particularly those that prey upon specific clades of hosts (Bagchi *et al.* 2010; Novotny *et al.* 2010). Phylogenetic relatedness interacted with seedling height, such that larger seedlings suffered disproportionately high mortality when surrounded by closely related neighbours (Fig. 1b). This suggests that the detrimental effects of growing in closely related neighbourhoods accumulate through time, perhaps as a result of increasing populations of natural enemies, in accordance with recent experimental work (Mangan *et al.* 2010). Because natural enemies cannot directly detect shared evolutionary history, we interpret these patterns as arising from the responses of natural enemies to phylogenetically correlated functional traits.

The observed positive relationship between phylogenetic relatedness and seedling mortality could potentially be generated by within-species mechanisms. More precisely, the relationship could occur intra-specifically if mortality rates were elevated among seedlings growing in dense monospecific stands (so-called 'seedling carpets'), especially among common species (Swamy & Terborgh 2010; Alvarez-Loayza & Terborgh 2011). Since mortality of only five of the 20 most-common species was significantly related to conspecific density (Appendix S2), it appears that phylogenetic relatedness, rather than conspecific density, generated the patterns we observed.

We evaluated phylogenetic relatedness in this study using a newly generated community phylogeny, which allowed us to determine whether natural enemies respond more strongly to ecological divergences associated with relatively ancient or recent nodes. Most previous studies have assessed phylogenetic relatedness with trees of global scope but only family- or genus-level resolution (Webb *et al.* 2006; Metz *et al.* 2010; but see Gonzalez *et al.* 2010 for an exception), whereas our tree had species-level resolution in most clades. In this study, we detected similar phylogenetic patterns when using trees resolved to family or species level. Our results suggest that natural enemies respond most strongly to ecological differentiation associated with ancient divergences in the angiosperm phylogeny, which are retained in trees with or without species-level resolution. Although far from conclusive, this observation is consistent with an interpretation that defensive chemistry plays a strong role in the selection of host plants, since many of the important evolutionary divergences in defensive chemistry occur in the angiosperm phylogeny at or above the taxonomic level of families (Becerra 1997; Fine *et al.* 2006). Nevertheless, natural enemies participate in ongoing arms races within at least some of the families and genera we studied (Fine *et al.* 2006; Kursar *et al.* 2009). Both clade- and community-scale approaches will be necessary to further disentangle the mechanisms generating NDD in these forests.

The effects of stem density

As in most previous studies, total seedling density was not a significant predictor of seedling mortality (Comita *et al.* 2010; Metz *et al.* 2010; but see Webb *et al.* 2006). But surprisingly, and in contrast to previous findings (e.g. Comita *et al.* 2010; Metz *et al.* 2010), elevated conspecific stem density was associated with increased mortality rates in only five of the 20 most-common species. This suggests several possibilities: that there are relatively few host-specific natural enemies in this forest, that they are not causing seedling mortality, or that they are not acting in a density-dependent manner. Owing to distance-limited seed dispersal, dense stands of conspecific seedlings could occur in the

proximity of conspecific adult trees, potentially intensifying adult-seedling interactions. The effects of adults may be discounted here, however, because in the current study seedling density was uncorrelated with the distance to conspecific adults ($R^2 = 0.007$). Had we studied younger seedlings, or predation on seeds, this correlation might have been more substantial. Finally, host-specific pathogens may increase mortality rates in high-density patches of conspecific seedlings (Alvarez-Loayza & Terborgh 2011). Our finding that the mortality rates of one-quarter of the most common species were sensitive to conspecific seedling density is in accord with an entomological study from Guyana, where 16 and 25% of sap-sucking and leaf-chewing insects, respectively, were host specialists (Basset 1999). The effects of conspecific density on seedling mortality may increase with rarity (Comita *et al.* 2010). A full analysis of the potentially interactive effects of rarity and conspecific density was beyond the scope of this study, however, owing in part to the enormous sample sizes necessary to estimate mortality rates for rare species.

The effects of adult trees

Neighbouring adults had relatively minor effects on seedling mortality, compared to neighbouring seedlings (Fig. 1 and Table 2). This stands in contrast to previous results, where the basal area of conspecific adults and the density of conspecific seedlings both increased seedling mortality (Janzen 1972; Metz *et al.* 2010). Our finding suggests that the importance of adult trees as reservoirs of natural enemies is less than has been previously suggested, at least in this forest. This is consistent with recent research showing that most insect herbivores that feed on seedlings are not specialists that originate on neighbouring conspecific adults (reviewed in Basset *et al.* 2003). Our definition of 'adult' trees (i.e. DBH ≥ 10 cm) may include pre-reproductive individuals of large-statured species as well as males of dioecious species. Had we more accurately classified adult status, a more distinct effect of adults on seedling mortality may have become evident. Furthermore, many herbivorous arthropods, such as gall-makers and leaf-miners, prefer certain strata of the forest (i.e. canopy or understory, Basset *et al.* 2003). It may be the case that other natural enemies, such as fungi and pathogenic bacteria also prefer specific strata, given the massive changes in environmental conditions among strata. Substantial efforts would be necessary to determine habitat associations for a diverse array of natural enemies (Alvarez-Loayza & Terborgh 2011).

CONCLUSIONS

Negative density dependence and environmental filtering contribute to community assembly. The former promotes long-term species coexistence by allowing species to recover from low population density, whereas the latter eliminates individuals with traits unsuited to the local conditions from the community. NDD, most likely generated by natural enemies, appeared to affect community assembly more strongly and more pervasively than did EF. It appears that functional traits associated with the leaf economics spectrum have little effect on host selection; rather, we infer that natural enemies may select host plants on the basis of phylogenetically correlated functional traits. This tentative inference is based on the elimination of alternative hypotheses, however, and awaits experimental demonstration. Jointly, these processes contribute to community assembly in this diverse neotropical rain forest. These results highlight the prominent role of

stabilizing mechanisms of species coexistence in maintaining the diversity of species-rich ecosystems, while leading towards a mechanistic understanding of community assembly.

AUTHOR CONTRIBUTIONS

CETP, PMF and CB designed the study. All authors collected data. CETP analysed the data and wrote the article with contributions from all authors.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Details of the phylogenetic tree reconstruction.
Appendix S2 Results from supplemental analyses.

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