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Community ecology

The effects of rainforest fragment area on the strength of plant–pathogen interactions

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Pathogenic interactions between fungi and plants facilitate plant species coexistence and tropical rainforest diversity. Such interactions, however, may be affected by forest fragmentation as fungi are susceptible to anthropogenic disturbance. To examine how fragmentation affects fungus-induced seed and seedling mortality, we sowed seeds of six plant species in soils collected from 21 forest fragments. We compared seedling establishment in unmanipulated soils to soils treated with fungicides. Fungicides increased germination of *Toona ciliata* seeds and decreased mortality of *Syzygium rubicundum* and *Olea dioica* seedlings. The fungus-induced mortality of one of these species, *S. rubicundum*, decreased with decreasing fragment size, indicating that its interactions with pathogenic fungi may weaken as fragments become smaller. We provide evidence that a potential diversity-maintaining plant–fungus interaction weakens in small forest fragments and suggest that such disruptions may have important long-term consequences for plant diversity. However, we emphasize the need for further research across rainforest plant communities to better understand the future of diversity in fragmented rainforest landscapes.

1. Introduction

Plant–pathogen interactions play crucial roles in tropical forests [1]. In particular, recent studies [2,3] suggest that fungal pathogens may be especially important for the maintenance of plant diversity in rainforests by mediating negative density-dependence [4–6] and plant–soil feedbacks [7]. We know little, however, about how anthropogenic perturbations, like forest fragmentation, affect interactions between plants and their fungal pathogens [8,9], and consequently plant diversity. Such understanding is particularly relevant given the large proportion of tropical rainforests threatened by fragmentation [10,11]. Fragmented forests contain fewer species than similar areas of contiguous forest [10,11], but provide important reservoirs of biological diversity in the absence of contiguous forest [12]. The long-term future of this biodiversity may depend on the resilience of processes that maintain plant diversity, like those mediated by pathogenic fungi, to environmental changes like forest fragmentation. Fragmentation can lead to lighter, warmer and drier microclimates [10,11,13], which may reduce soil fungal biomass [14], weaken plant–soil feedbacks [7] and inhibit fungal pathogen transmission and infection [7,9,15,16]. Fragmentation may also alter fungal species composition and lower fungal diversity, although the effects on fungal

Table 1. Results of mixed effects Cox proportional hazards models examining the effects of fungicide and fragment size on germination. Significant effects ($p < 0.05$) in italics.

	Syzygium rubicundum		*Olea dioica*		*Symplocos racemosa*	
family	Myrtaceae		Oleaceae		Symplocaceae	
N	events | total: 357|570		events | total: 333|550		events| total: 390|555	
random effects:	***N***	**var**	***N***	**var**	***N***	**var**
site	21	0.02058	21	0.10294	21	0.01920
fixed effects:	**est.**	**s.e.**	**est.**	**s.e.**	**est.**	**s.e.**
fungicide	0.002	0.32	−0.274	0.50	−0.128	0.31
fragsize	0.122	0.16	0.163	0.33	0.011	0.23
fragsize: fungicide	−0.019	0.13	−0.002	0.13	−0.060	0.14
	Syzygium cumini		*Syzygium gardneri*		*Toona ciliata*	
family	Myrtaceae		Myrtaceae		Meliaceae	
N	events | total: 353|504		events | total: 313|440		events | total: 175|436	
random effects:	***N***	**var**	***N***	**var**	***N***	**var**
site	21	0.00008	21	0.00002	21	0.00008
fixed effects:	**est.**	**s.e.**	**est.**	**s.e.**	**est.**	**s.e.**
fungicide	−0.633	0.43	−0.398	0.48	*0.537*	*0.19*
fragsize	−0.180	0.25	−0.382	0.29	0.117	0.17
fragsize: fungicide	0.257	0.17	0.173	0.16	−0.252	0.21

pathogens vary widely [17]. Consequently, ecological processes dependent on fungi, like plant–soil feedbacks, may be disrupted in fragmented landscapes.

To understand how fragmentation modifies plant–pathogen interactions, we investigated whether fragment size influences the effects of soil-borne fungal pathogens on seedlings of six rainforest tree species. We focused on soil-borne fungi because their ability to influence plant populations through plant–soil feedbacks has been previously highlighted [3]. As drier microclimates typical of smaller fragments [13] are less conducive for soil-borne fungi [14], we hypothesized that fungus-mediated seedling mortality would decrease as fragments become smaller. We tested our hypothesis in a shadehouse experiment by comparing plant performance in rainforest soils collected from a gradient of fragment sizes under two treatments (fungicide-treated and control).

2. Material and methods

This study was conducted in Kadamane Tea Estate (12.8639°–12.5620° N and 75.6361°–75.6833° E) in the Western Ghats, Karnataka, India. This 30 km^2 private estate is a mosaic of fragmented rainforest, high elevation grassland and cultivated tea.

We collected soil samples (approx. 0.06 m^3 each, approx. 0–30 cm deep including leaf litter) from 21 fragments 1–149 ha in area, with small and large fragments interspersed through the site (electronic supplementary material, figure S1, mean fragment area 27.8 ha, elevation 918–1071 m). Samples were collected from 30 random locations within a comparable area (more than 40 m from any edge) within each fragment and pooled and homogenized to obtain a single representative source of soil-borne fungi for each forest fragment. Soil from each fragment was placed in twelve 20 × 10 × 10 cm trays after equal and random distribution between 'control' and 'fungicide' treatments. All trays were kept in a 75% shaded greenhouse constructed of mesh that allowed continuous homogeneous percolation of rainwater but excluded large insects. Soils from small and large fragments were well interspersed in the shadehouse. No insect herbivory was observed inside the greenhouse.

We collected seeds of six tree species (electronic supplementary material, table S1), discarding those that showed evidence of predispersal predation by insects or that floated in the water. We sowed 4–5 seeds (depending on availability) of each species in each of 126 trays (3 replicates of 4–5 seeds/treatment/fragment). We monitored germination and seedling mortality at five censuses 15–30 days apart between 23 May and 9 October 2017, or until most seedlings of a species grew four open leaves and lost their cotyledons (electronic supplementary material, table S1).

We combined a systemic fungicide *Azoxystrobin* (Product name: Amistar®, Syngenta, Basel, Switzerland; 0.4 ml l^{-1} water), a contact fungicide *Mancozeb* and an oomyticide *Metalxyl* (Product name: Ridomil Gold® Syngenta; 6 g l^{-1} water) to exclude fungi and oomycetes. We sprayed approximately 8 ml of diluted Amistar® and 8 ml of diluted Ridomil Gold® on the trays in the fungicide treatment every eight days (dry season), and every 4 days (wet season, to account for immediate runoff in heavy rain). To control for effects of supplemental water, we sprayed trays in the control treatment with a volume of water equal to that used to dissolve the fungicides.

Using mixed-effects Cox proportional hazards models, we modelled time to germination or mortality as functions of fungicide treatment, fragment size and their interaction and included seedling tray as a random effect. We analysed the germination of all six species individually but analysed the seedling mortality of only three species, with sufficient dead seedlings, individually. In a separate model, we combined data from the remaining three species. We 'censored' seeds that did not germinate in the germination analysis and seedlings that survived in the mortality analysis. Models were fitted using the coxme package [18] from R 3.4.0 [19]. Statistical assumptions were confirmed using model

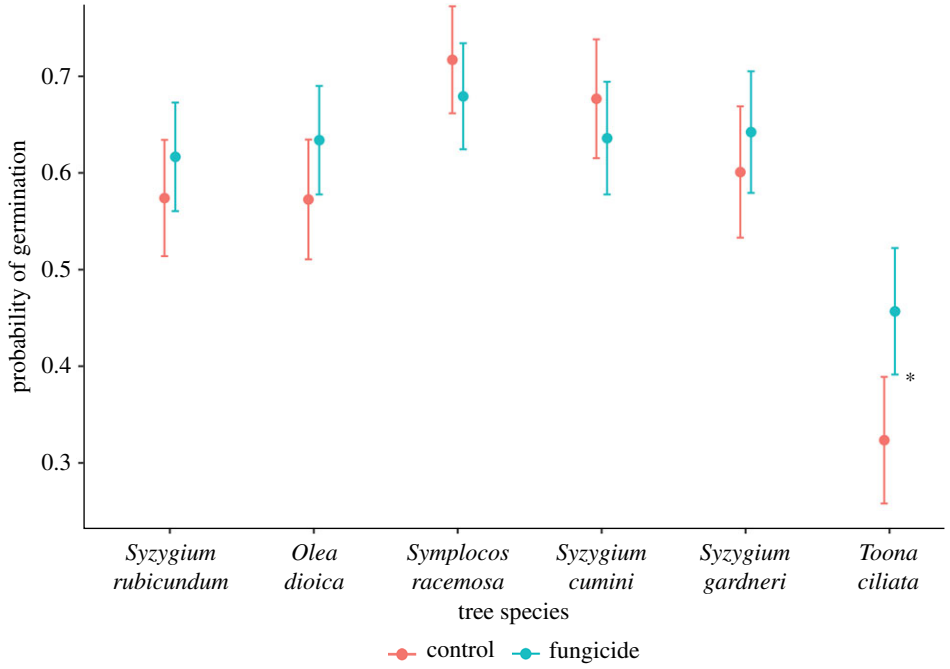

Figure 1. Effects of fungicide on the seed germination probabilities of six rainforest tree species. Observed values are plotted with 95% confidence intervals. The asterisk represents a significant effect ($p < 0.05$).

Table 2. Results of mixed effects Cox proportional hazards models examining the effects of fungicide and fragment size on seedling mortality. Significant effects ($p < 0.05$) in italics.

	Syzygium rubicundum		*Olea dioica*		*Symplocos racemosa*		other three species	
N	events |total: 40|322		events |total: 64|313		events |total: 53|357		events |total: 35|744	
random effects:	***N***	**var**	***N***	**var**	***N***	**var**	***N***	**var**
site	21	0.0004	21	0.0771	21	0.2553	21	0.7418
fixed effects:	**est.**	**s.e.**	**est.**	**s.e.**	**est.**	**s.e.**	**est.**	**s.e.**
fungicide	−0.30	0.43	*−0.69*	*0.32*	0.21	0.36	0.15	0.41
fragsize	*0.46*	*0.16*	−0.05	0.19	0.41	0.28	0.21	0.44
fragsize: fungicide	*−1.18*	*0.57*	−0.28	0.36	−0.25	0.34	−0.40	0.49

diagnostics and consistency with alternative models (electronic supplementary material, table S2 and figure S2).

3. Results

Of the 3483 seeds of six species, 2171 germinated, of which 258 subsequently died. Fungicide treatment increased germination for *Toona ciliata* (table 1 and figure 1) and decreased seedling mortality for *Syzygium rubicundum* and *Olea dioica*. (table 2 and figure 2). Germination and survival of the other three species were unaffected by fungicide.

Germination was statistically independent of fragment size for all species (table 1). Seedling mortality of *S. rubicundum* increased with increasing fragment size in the control treatment, but fungicide treatment removed that relationship (table 2 and figure 2). Mortalities of the remaining species were unaffected by fragment size in both control and fungicide treatments.

4. Discussion

Suppressing soil-borne fungi influenced the survival of three plant species, but fragment size only influenced the effects of fungi on *Syzygium rubicundum*. Seedling mortality of *S. rubicundum* increased strongly with fragment size in the control treatment, but this effect was removed completely when fungi were suppressed. Our results for this one species, therefore, support the hypothesis that mortality owing to fungi may be affected by perturbations from forest fragmentation.

Given that the performance of species other than *S. rubicundum* was independent of fragment size, the wider implications of our results for how soil-borne fungal pathogens shape tree-communities in forest fragments remain unclear. Although fungi negatively influenced *T. ciliata* and *O. dioica* (consistent with [20]) in addition to *S. rubicundum*, interactions between fungicide and fragment size were limited to *S. rubicundum*. Positing a general mechanism for attenuation of phytopathogen infections in small forest fragments would, therefore, be speculative. Soils collected from under adult conspecifics may have inhibited the survival of more species or led to stronger relationships with fragment size. The most probable explanation for the variation among plant species is that responses of pathogenic fungi to fragmentation are very variable [17]. Such variability might mean that the community-wide implications of altered plant–pathogen interactions require

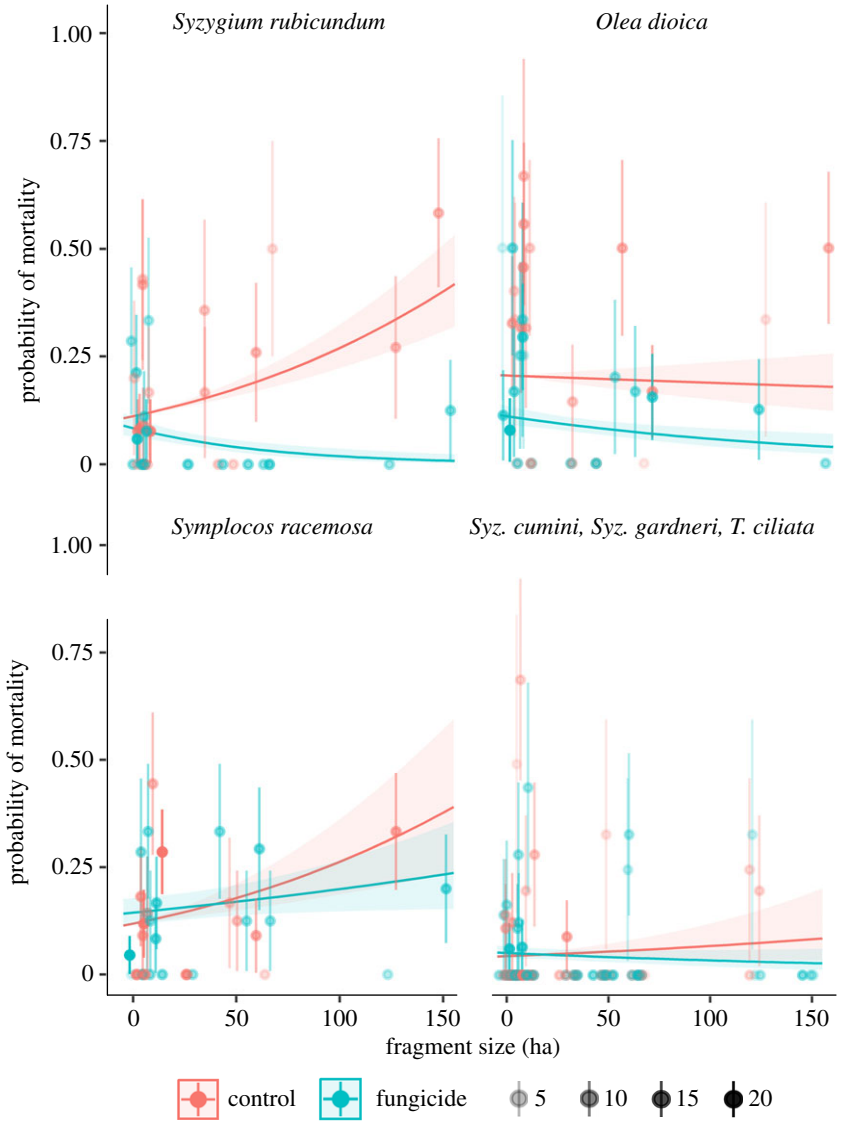

Figure 2. Effects of fungicide and fragment size on the seedling mortality probabilities of six rainforest tree species. Observed values are plotted with standard errors. Lines and standard errors are predicted from the models in table 2. Degree of shading represents the number of seedlings in a sample.

integration over large numbers of species, perhaps combined using simulation-based approaches.

An alternative explanation for the observed relationship between fragment size and survival in *S. rubicundum* is a positive correlation between *S. rubicundum* density and fragment size. We found that fragment size was uncorrelated with *S. rubicundum* density as seeds, seedlings and fruiting adults (see electronic supplementary material), and consequently infer that this explanation is unlikely. It is important to note that *S. rubicundum* was the most abundant species in the seedling assemblage at the site. That the mortality of this dominant tree species was reduced in soils from smaller fragments presents a critical question—to what extent can the responses of abundant species to fragmentation have cascading effects on the rest of the plant community? If *S. rubicundum* and other abundant species are released from fungal control, their dominance of the community may increase within small fragments, potentially decreasing plant biodiversity over time.

Our results join a growing body of literature indicating that fungal–plant interactions are sensitive to various forms of anthropogenic disturbance including fragmentation [9,17], edge effects [8,20] and simulated drought [16,21]. These results may represent early warnings that fungal–plant interactions are disrupted in human-modified tropical forests. Given the importance of such interactions especially for the maintenance of plant diversity [2,3,22], such disruption could have profound implications for the future of remnant tropical forest communities. The consequences of these altered interactions at the wider community and ecosystem scales, however, have been largely unexplored, but present an important direction for future research.

Data accessibility. Data available from the Dryad Digital Repository: https://dx.doi.org/10.5061/dryad.6nc5572 [23].

Authors' contributions. A.V., R.B. and J.G. conceived the study, which was designed by A.V., G.H. and N.A.K.; A.V., G.H. and N.A.K. collected data. A.V. performed statistical analyses with input from R.B. A.V. drafted the manuscript with input from J.G. and R.B. All authors contributed to manuscript editing, gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests. We declare we have have no competing interests.

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