LETTER

Experimental evidence for a phylogenetic Janzen–Connell effect in a subtropical forest

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Abstract

Observational evidence increasingly suggests that the Janzen–Connell effect extends beyond the species boundary. However, this has not been confirmed experimentally. Herein, we present both observational and experimental evidence for a phylogenetic Janzen–Connell effect. In a subtropical forest in Guangdong province, China, we observed that co-occurring tree species are less phylogenetically related than expected. The inhibition effects of neighbouring trees on seedling survival decreased with increasing phylogenetic distance between them. In a shade-house experiment, we studied seedling survival of eight species on soil collected close to *Castanopsis fissa* relative to their survival on soil close to their own adult trees, and found that this relative survival rate increased with phylogenetic distance from *C. fissa*. This phylogenetic signal disappeared when seedlings were planted in fungicide-treated soil. Our results clearly support negative effects of phylogenetically similar neighbouring trees on seedling survival and suggest that these effects are caused by associated host-specific fungal pathogens.

Keywords

Fungi, host specificity, pathogens, phylogenetic distance, seedling survival, species coexistence, subtropical forest.

INTRODUCTION

The extraordinarily high plant diversity in the tropics has fascinated ecologists for decades. Various theories have been proposed to explain the mechanisms that regulate abundances and coexistence of different species (e.g. Grubb 1977; Tilman 1982; Taylor *et al.* 1990; Hubbell 2001). One of them is the Janzen–Connell hypothesis (Ridley 1930; Janzen 1970; Connell 1971), which has gained considerable empirical support (see reviews in Hammond & Brown 1998; Wright 2002; Freckleton & Lewis 2006). The Janzen–Connell hypothesis states that offspring located close to adult conspecifics suffer increased mortality caused by natural enemies (herbivores and pathogens). Therefore, offspring of heterospecific species have a competitive advantage, leading to higher diversity.

Fungal pathogens, particularly soil-borne oomycetes causing damping-off diseases of young seedlings (Cohen & Coffey 1986), have been found to drive density- and/or distance-dependent seedling mortality in tropical forests (Augspurger 1983, 1984; Augspurger & Kelly 1984; Dalling *et al.* 1998; Gilbert 2002; Hood *et al.* 2004; Bell *et al.* 2006; Mangan *et al.* 2010) as well as in temperate forests (Packer & Clay 2000; Reinhart *et al.* 2003; Reinhart & Clay 2009). It also has been demonstrated that soil material collected close to parent trees (e.g. at a distance of 0–5 m) has a strong negative effect on survival of conspecific seedlings (Packer & Clay 2000, 2003; Li *et al.* 2009).

The Janzen–Connell hypothesis assumes that natural pathogens or herbivores are host species-specific. However, recent studies reported that pathogens and herbivores are rarely monophagous and usually attack several closely related hosts (e.g. Novotny *et al.* 2002; Agrawal & Fishbein 2006; Gilbert & Webb 2007). The great phylogenetic variation of species in a forest would be ignored by simply separating neighbourhood individuals around a given species into conspecifics or heterospecifics (Pacala *et al.* 1996). A more realistic assumption is that closely related neighbours have a greater negative influence on each other than neighbours that are more distantly related. Aided by the increasing availability of phylogenetic data, some authors have therefore extended the Janzen–Connell hypothesis with respect to the evolutionary distance between two neighbouring species in a tree community (Webb *et al.* 2002, 2006; Novotny *et al.* 2002; Weiblen *et al.* 2006; Gilbert & Webb 2007; Bagchi *et al.* 2010; Metz *et al.* 2010; Ness *et al.* 2011; review by Cavender-Bares *et al.* 2009). For example, Webb *et al.* (2006) analysed the relationship between seedling survival and phylogenetic neighbourhood effects in a tropical rain forest. They found that seedling survival was enhanced when surrounding seedlings in the same quadrat were phylogenetically more distantly related. They further suggested that positive effects on seedling survival could be related to interactions with pathogens. By experimental inoculation of plant leaves with fungal pathogens in a tropical rain forest, Gilbert & Webb (2007) found that the likelihood that a pathogen can infect two different plant species decreases with increasing phylogenetic distance. However, these studies do not provide experimental evidence of how the evolutionary relatedness of adults affects the survival of nearby seedlings. Pathogen exclusion experiments are required to clearly demonstrate a host-specific influence of associated pathogens on seedling survival.

Herein, we report observational and experimental results. The main aim of this study was an experimental test of the phylogenetic Janzen–Connell effect, but before performing such a test, we first carried out a field study as a preliminary study to the experimental work to see if there is an indication of the Janzen–Connell effect in the field. We conducted a field census in a subtropical forest where we investigated

the extent to which different evolutionarily related neighbouring trees affect seedling survival in a natural tree community. We then experimentally studied seedling survival of *Castanopsis fissa* (Fagaceae) trees and seven other tree species that varied in their phylogenetic distance to *C. fissa* (Fig. 1), on soil collected at a close distance to adult *C. fissa* and on soil collected close to the other tree species. We found that the ratio of survival on *C. fissa* soil and on the species' 'own' soil increased with phylogenetic distance to *C. fissa*. This effect was strongly reduced after fungicide treatment of the soil.

MATERIALS AND METHODS

Study site

We conducted field work at Heishiding Nature Reserve (111°53′ E, 23°27′ N, 150–927 m altitude), in Guangdong Province, China. The reserve consists of approximately 4200 ha of subtropical evergreen broad-leaved forest, and the Tropic of Cancer runs through its centre. The region has a subtropical moist monsoon climate. Mean annual temperature is 19.6 °C and mean monthly temperatures range from 10.6 °C in January to 28.4 °C in July. Annual precipitation is about 1743.8 mm, occurring mainly between April and September (79% of annual rainfall), and a pronounced dry season lasts from October to March. Species belonging to the Fagaceae and Lauraceae families, which are broadly distributed in subtropical evergreen broad-leaved forests, are the dominant tree species in this region.

Field surveys

To estimate the phylogenetic structure of subtropical forest tree communities and to investigate the effect of different neighbouring tree species on seedling survival in natural communities, we established six 1 ha permanent plots during winter 2007 to spring 2008. These plots are representative for subtropical evergreen broad-

Figure 1 Phylogenetic relationships of tree species used for the shade-house experiment. Numbers show relative branch lengths (on a scale from 0 to 1) calculated using Phylocom (Webb *et al.* 2008). Germinated seedlings of these species were planted into the soil substrate, which was collected under adult *Castanopsis fissa* or conspecific trees.

leaved forests; three of them are located at relatively high altitude (600 m above sea level), and the other three at low altitude (340 m). All saplings and adult trees with a diameter at breast height (DBH) ≥ 1 cm were analysed in the plots. Their diameter was measured, their position was mapped and their species names were determined. In total, 181 species from 106 genera and 55 families were identified, comprising about 29 800 individuals. We did a statistical comparison using the Simpson index to test whether the altitude affects species composition: the Simpson index (mean ± SD) is 0.92 ± 0.06 when two individuals are drawn from two different altitudes, and it equals 0.89 ± 0.05 ($P = 0.22$) or 0.85 ± 0.09 ($P = 0.08$) when the two are drawn from only low or high altitude respectively. These results indicate that if we take two individuals from different altitudes, they are just as likely to be the same species as two individuals from the same altitude. Hence, we combined the two altitudes in the subsequent analysis.

In spring 2008, we demarcated 1200 1×1 m^2 test quadrats, regularly located within each quadrats, and all seedlings of woody plants (DBH < 1 cm) were surveyed (see Supplementary Figure S1 for details of seedling quadrat network design). The annual census was repeated in spring 2009, 2010 and 2011 respectively. We analysed the first-year survival of newly geminated seedlings. As direct neighbourhood interactions in tree communities usually are believed to occur at a radius distance of 20–30 m (Hubbell *et al.* 2001; Peters 2003; Stoll & Newbery 2005; Comita *et al.* 2010), we excluded the seedling quadrats with a distance of < 30 m to any side of the plot from the analysis (see Figure S1 for details). For each focal seedling, we determined the sum of the basal area of conspecific individuals and the standardised effect size of mean phylogenetic distance (described below) with all trees (≥ 1 cm DBH) within 10 m interval rings from 0 to 30 m (Comita *et al.* 2010; Metz *et al.* 2010). Using data of 1311 seedlings in 240 seedling quadrats which were newly geminated in 2008, 2009 and 2010, we examined the relationship between the probability of seedling survival and the phylogenetic relatedness of adult neighbouring trees.

Phylogenetic data

We obtained the topology of the phylogeny for the 181 tree species occurring in the permanent plots with the Phylomatic program (Webb & Donoghue 2005), which is based on the APG III (Angiosperm Phylogeny Group [APG] 2009) derived megatree (version R20091110.new). We then used the BLADJ algorithm of the Phylocom version 4.1 software package (Webb *et al.* 2008) to obtain an ultrametric tree with branch lengths scaled to time, based on clade ages according to Wiksrtöm *et al.* (2001).

The six 1 ha permanent plots were divided into non-overlapping square quadrats of five different spatial scales: 10×10 m, 20×20 m, 30×30 m, 50×50 m and 100×100 m. We used Webb's (Webb 2000; Webb *et al.* 2002) net relatedness index (NRI) and nearest taxon index (NTI) to measure the phylogenetic structure of these quadrats. NRI has been proposed as a measure of tree-wide phylogenetic clustering and overdispersion of species (the mean relatedness of all heterospecifics), and NTI has been proposed as a measure of terminal phylogenetic clustering and overdispersion (Webb 2000). Positive NRI and NTI values indicate that species occurring together in a quadrat are more closely phylogenetically related than expected by chance (clustering), and negative NRI and NTI values indicate that co-occurring species are less phylogenetically related than expected by
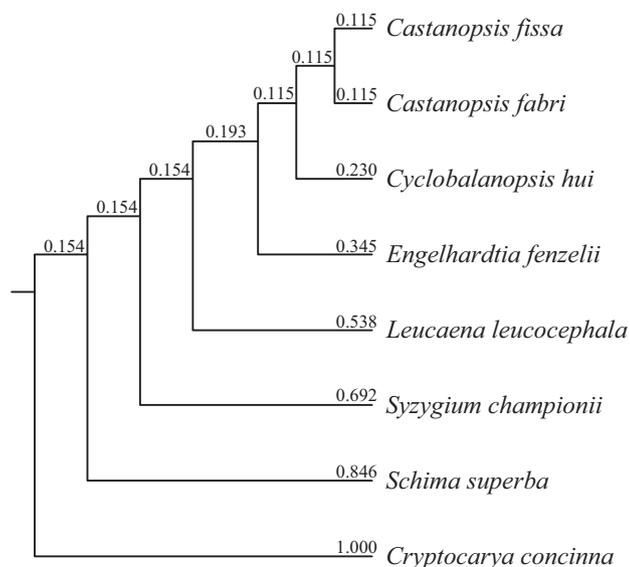
chance (overdispersion). The null model randomizes according to the independent swap algorithm maintaining species occurrence frequency and sample species richness, where the species pool consists of all 181 species found in our six 1 ha plots. The NRI and NTI of all quadrats at a given spatial scale were calculated using the package 'picante' in R for Windows 2.13.0 (Kembel *et al.* 2010; R Development Core Team 2011).

Phylogenetic distances between all pairs of species occurring in the permanent plots were calculated using the PHYDIST procedure of Phylocom (Webb *et al.* 2008). According to these distances, we calculated the mean phylogenetic distance between each focal seedling and all other species in the adult neighbourhood using summed phylogenetic distances. The mean phylogenetic distance was then standardised by the mean expected phylogenetic distance, given a specified number of species in the same neighbourhood, to correct the variance of phylogenetic mean distance with the species richness of the sample (Webb *et al.* 2006). The null model used for the standardisation here is obtained in the same way as in the test of phylogenetic clustering/overdispersion using the NRI and NTI. Negative mean phylogenetic distance values indicate that the neighbourhood plants are less related to the focal seedling than expected from the null model, and positive values indicate that the neighbourhood plants are more related than expected.

Shade-house experiment

Eight tree species commonly occurring at the field study site were selected: *Castanopsis fissa* Rehd. et Wils (Fagaceae), *C. fabri* Hance (Fagaceae), *Cyclobalanopsis hui* Chun (Fagaceae), *Engelhardtia fenzelii* Merr. (Juglandaceae), *Leucaena leucocephala* de Wit (Leguminosae), *Syzygium championii* Merr. et Perry (Myrtaceae), *Schima superba* Gardn. et Champ. (Theaceae) and *Cryptocarya concinna* Hance (Lauraceae). These species were chosen because their phylogenetic distance to *C. fissa* increased approximately evenly, resulting in a series of species pairs whose evolutionary relatedness gradually decreases (Fig. 1).

We used experimentally germinated seedlings to evaluate the effect of soil and fungicide on the different evolutionarily related species. We performed experimental treatments in a shade-house at the field site using the following variables: (1) phylogenetic distance (between *C. fissa* and each of the other species), (2) soil source (soil collected close to adult *C. fissa* trees or adult trees of the other species), and (3) fungicide treatment (applied to soil material; an equivalent volume of water was added to the control plots). We applied the metalaxyl-based fungicide Captan WP (Pangu Ltd, Changzhou, China), which can provide broad-spectrum protection against fungi and oomycetes (Cohen & Coffey 1986), to the soil surface of fungicide-treat pots. We measured the soil respiration rates for both fungicide soil and control soil of each species using SRS-2000 Portable Soil Respiration System (ADC BioScientific, Hertfordshire, UK), to check whether the fungicide actually influenced soil microbial activity. We performed the fungicide treatment to check whether the observed negative effects of neighbouring trees on seedling survival are caused by soil-borne pathogenic fungi.

We collected fruits and seeds from trees throughout the study site during autumn and winter 2009. Seeds were surface-sterilized (1 min 70% ethanol, 3 min 50% commercial bleach, 1 min 70% ethanol, 1 min distilled water) and kept in a refrigerator at 4 °C until late March 2010. Seeds were left to germinate in plastic boxes filled with moistened sterilised sand. Seeds of all eight species started to germinate during the following 4 weeks.

We chose 12 adult *C. fissa* trees and three adult trees of each of the other species as inoculum sources. We collected soil at a depth of 0–30 cm and at a distance of 0–2 m beneath these adult trees in late April 2010 and thoroughly mixed it for each species. We filled plastic pots (25 cm in diameter, 21 cm in height) with this soil and transferred the germinated seedlings into these pots; we planted seedlings of each species into both *C. fissa* soil pots and their own soil pots (*L. leucocephala* seedlings were only planted into *C. fissa* soil because of insufficient seeds). The *C. fissa* soil pots also had density treatments (four seedlings per pot for a low-density treatment and 12 for a high-density treatment), and the conspecific soil pots only had high-density treatment (12 seedlings per pot). One week after transfer of germinated seedlings into pots, we removed the seedlings that were dead or poorly growing due to injuries during the transfer, and we replaced them with new seedlings.

The experimental units comprised 15 blocks, each of which was divided into two sub-blocks. All sub-blocks were separated by a distance of 0.5 m to limit the potential drift of fungicide. Pairs of neighbouring sub-blocks in the same block were either sprayed with fungicide or mock-treated with water. We applied the fungicide Captan WP using a portable waterpot according to the manufacturer's recommendations (2.5 g m^{-2}), with 50 mL of solution applied to the soil surface of each pot ($\approx$ 0.05 m^2). We treated control pots with an identical volume of water. We repeated the fungicide treatment every 2 weeks, regularly watered the seedlings and monitored seedling survival weekly. All seedlings were allowed to grow for 5 months. At the end of the experiment, we harvested seedlings and determined their total dry weight. We also measured the contents of NPK in the soil of all species to test whether they were influenced by the fungicide treatment.

Statistical analysis

For the observational data, to test whether the average phylogenetic structure of local tree communities at a given spatial scale differed from random, we determined at each spatial scale the mean phylogenetic structure to be the mean NRI and NTI of all quadrats. Using one-sample t tests to see if the mean NRI and NTI differed from zero at a certain spatial scale, we determined how close the average phylogenetic structure was to total randomness (Kembel & Hubbell 2006).

We analysed the first-year survival for seedlings that geminated in 2008, 2009 and 2010 with a generalised linear mixed-effects model (GLMM), in which we entered conspecific tree abundances (log-transformed) and the mean phylogenetic distances of neighbouring adults as fixed factors, assuming a binomial error and logit-link function. We also included each seedling's recruitment year and location (1 m^2 quadrat) as factors in the model, to account for potential spatial and temporal autocorrelation (Metz *et al.* 2010). The estimated coefficients of the model present a measure of the strength of neighbour effects.

We used odds ratio regression to compare the effects of experimental treatments on seedling survival of differently related species, and to determine whether the odds ratio was significantly correlated with phylogenetic distance. Odds ratio regression uses a maximum likelihood approach where the distribution of the log odds ratio is assumed to be approximately normal (Jewell 2004), and where a likelihood ratio test checks for significant departure from no relationship with phylogenetic distance. As the *C. fissa* soil pots had

low- and high-density treatments, and the conspecific soil pots only had high-density treatment, we used only high-density treatment of the *C. fissa* soil pots in the comparison with conspecific soil pots to determine the effects of soil and fungicide treatment. Then, we used only the *C. fissa* soil pots to show the effect of density treatment. We included experimental blocks and sub-blocks in all of the odds ratio regression models as covariates, and there were no significant effects of blocks and sub-blocks.

To determine the effect of the soil treatment on seedling survival, we compared the seedling survival between 'away' soil (collected under *C. fissa* trees) and 'own' soil (collected under conspecific trees). We calculated the survival odds ratio comparing seedling survival in *C. fissa* soil vs. survival in conspecific soil for all species in the control pots, and used odds ratio regression models to determine whether the odds ratio (i.e. the relative survival) was correlated with phylogenetic distance. To test whether the fungicide treatment removed the phylogenetic distance effect, we did the same analysis comparing survival in *C. fissa* soil vs. survival in conspecific soil for the fungicide-treated pots. To determine the effect of the fungicide treatment on seedling survival, we calculated the survival odds ratio comparing survival in fungicide-treated soil vs. survival in control soil, for both *C. fissa* soil and conspecific soil. To determine the effect of the density treatment, we calculated the odds ratio between low density and high density for both control and fungicide-treated pots in *C. fissa* soil. All statistical analyses were performed using R for Windows 2.13.0 (R Development Core Team 2011).

RESULTS

Field surveys

In the field census, the mean NRI values of all quadrats were significantly lower than zero when calculated at spatial scales from 10×10 m to 50×50 m, but not for 100×100 m quadrats (Table 1). The mean NTI values had a similar but weaker trend (Table 1). Large proportions of these quadrats at smaller spatial scales had negative NRI and NTI values, demonstrating that co-occurring species were less phylogenetically related than expected. The NRI and NTI should be positive between plots because they increased with increasing spatial scales and the mean values at larger scales did not differ from zero (Table 1), indicating that species coexistence at smaller spatial scales may be influenced more intensively by processes that negatively affect close phylogenetic relatives, such as density dependence.

The first-year survival decreased with conspecific adult basal area (Fig. 2a–c) and decreased with the evolutionary relatedness between a given seedling and its neighbouring tree species (Fig. 2d–f), indicating that the chance that a seedling survives through the first year decreases if surrounding adults are more closely related to it. In particular, the estimated coefficients ($\pm$ SE) of the GLMM on a seedling's first-year survival were -0.032 ± 0.017 ($P = 0.016$), -0.246 ± 0.015 ($P = 4.7 \times 10^{-4}$) and -0.302 ± 0.014 ($P = 2.1 \times 10^{-6}$) for conspecific adult basal area within 0–10 m, 10–20 m and 20–30 m respectively; the coefficients were -0.279 ± 0.030 ($P = 6.7 \times 10^{-9}$), -0.193 ± 0.015 ($P = 3.4 \times 10^{-6}$) and -0.211 ± 0.014 ($P = 8.5 \times 10^{-5}$) for standardised mean phylogenetic distance to adult neighbours within 0–10 m, 10–20 m and 20–30 m respectively. We found similar results when the effects of neighbouring trees on seedling survival were calculated for a distance of 0–30 m and 0–20 m as a whole, rather than 20–30 m and 10–20 m rings (Figure S2).

Shade-house experiment

Although seedling survival had great variation among different species (Fig. 3a,b), the shade-house experiment yielded a survival odds ratio (log-transformed) comparing seedling survival in *C. fissa* soil with survival in conspecific soil that increased with increasing phylogenetic distance (slope = 0.711, likelihood ratio test with slope = 0: $P < 0.001$; Fig. 3c). The log odds ratio comparing seedling survival in *C. fissa* soil and conspecific soil was not correlated with phylogenetic distance in fungicide-treated pots ($P = 0.247$; Fig. 3d). At zero phylogenetic distance in Fig. 3c,d, the error in the odds ratio comes from experimental error and other random sources of variation (*C. fissa* seedlings were in fact transplanted in *C. fissa* soil twice). When we did the odds ratio regression without the zero-distant treatment and forced the regression line through the zero-zero point, we found similar results (Figure S3).

The fungicide significantly reduced the soil respiration rates in fungicide-treated pots comparing with control pots, but did not influence the NPK contents of the soil (Table S1). Treatments with the fungicide Captan WP in the shade-house experiment increased survival of seedlings (Fig. 3). Seedlings planted in *C. fissa* soil that were treated with fungicide showed much higher survival probability for *C. fissa* and closely related species (Fig. 3e). The fungicide, however, did not significantly affect survival of species more distantly related to *C. fissa* (slope = -0.668, $P = 0.006$; Fig. 3e). The fungicide also increased survival rates for seedlings planted in conspecific soil, and these effects were similar among all eight species ($P = 0.162$; Fig. 3f).
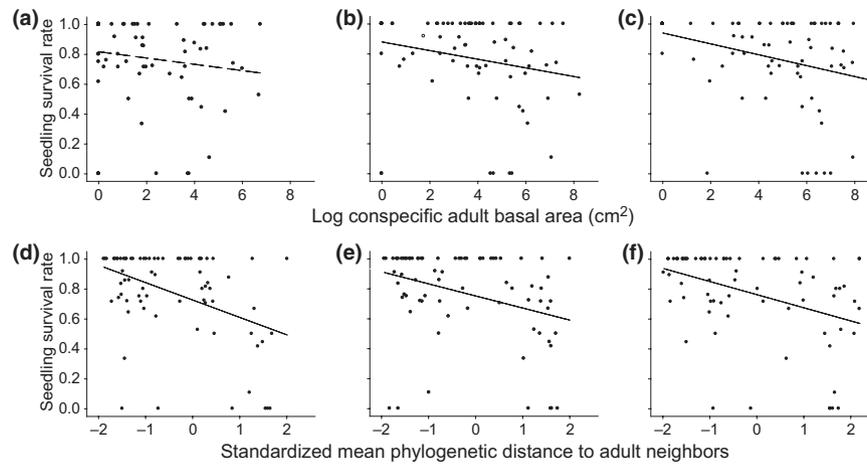
Table 1 Phylogenetic structure of tree communities in test quadrats at five spatial scales

Spatial scale (m)	Total quadrats N	Net relatedness index			Overdispersed quadrats		Nearest taxon index			Overdispersed quadrats	
		Mean*	SE	P†	N	percentage	Mean*	SE	P†	N	percentage
10×10	600	−0.702	0.035	<0.001	493	82.17	−0.216	0.035	<0.001	364	60.67
20×20	150	−0.707	0.062	<0.001	125	83.33	−0.175	0.058	0.003	89	59.33
30×30	54	−0.591	0.106	<0.001	41	75.93	−0.168	0.086	0.058	34	62.96
50×50	24	−0.470	0.164	0.009	17	70.83	−0.194	0.175	0.279	13	51.17
100×100	6	−0.304	0.278	0.325	4	66.67	0.051	0.209	0.818	3	50.00

*Negative net relatedness index (NRI) and nearest taxon index (NTI) values indicate that species occurring together in a quadrat are less phylogenetically related than expected by chance (phylogenetic overdispersion) and positive NRI and NTI values indicate phylogenetic clustering.
†Significant P-values indicate that the phylogenetic structure at a given spatial scale differed from zero according to a two-tailed *t* test.

Figure 2 Relationships between the first-year survival rates of newly germinated seedlings and (a–c) conspecific adult basal area or (d–f) mean phylogenetic distance to adult neighbours in the performed field survey. For each focal seedling, neighbouring trees located within 0–10 m for panels (a) and (d), 10–20 m for panels (b) and (e) and 20–30 m for panels (c) and (f) were analysed. Note that the mean phylogenetic distance is the mean distance of all neighbours to the focal seedling, and then standardised by the mean expected phylogenetic distance given a specified number of species (as in APd' by Webb *et al.* 2006); negative values indicate that the neighbourhood is less related to the focal seedling than expected from the null model, and positive values indicate that the neighbourhood is more related than expected. Points represent means for each species. Solid lines indicate linear regressions significant at $P < 0.05$, the dotted line indicates significance at $P < 0.1$.
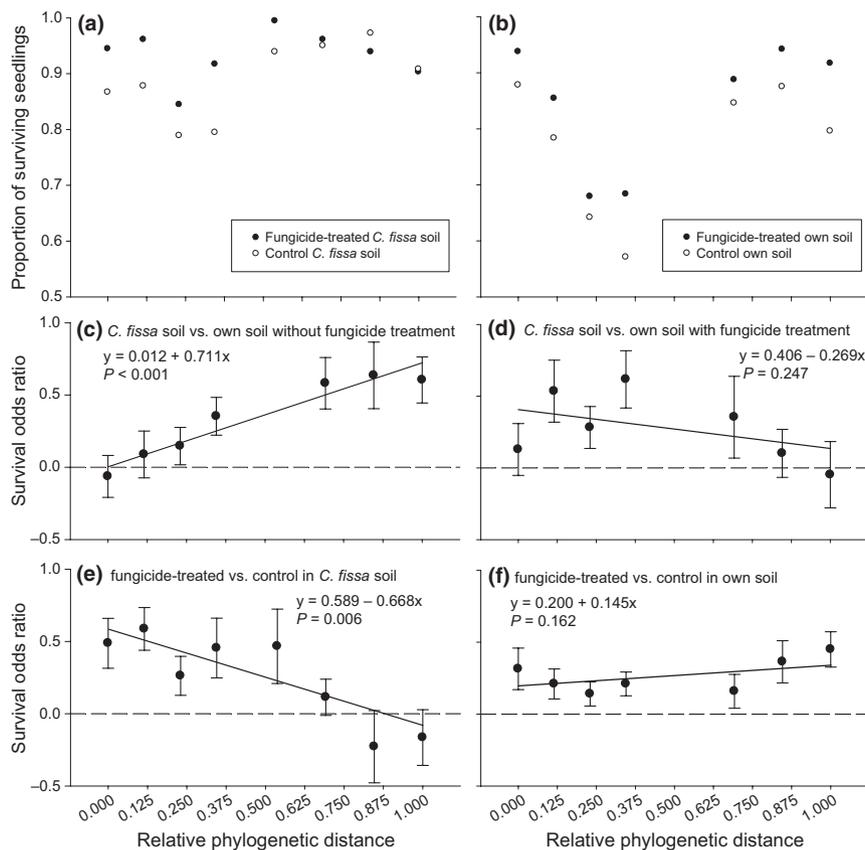

Figure 3 Effects of soil-borne fungi on seedling survival determined using a fungal exclusion experiment in a shade-house at the field site. Seedlings from different species were planted into pots filled with soil material obtained beneath adult *C. fissa* trees or conspecific trees. Pots were treated with Captan WP every 2 weeks. Effects of fungal pathogens are correlated with the phylogenetic distance between *C. fissa* and a given tree species. (a–b) Survival rate (proportion of surviving seedlings) of eight tree seedlings after growth for 5 months in fungicide-treated and control pots when seedlings were planted in (a) *C. fissa* soil or (b) conspecific soil. Each data point represents 180 and 240 seedlings for panel (a) and (b) respectively. (c–f) Effects of the soil treatment and fungicide treatment as a function of the phylogenetic distance: survival odds ratio between *C. fissa* soil vs. conspecific soil in (c) fungicide-untreated pots or (d) fungicide-treated pots; survival odds ratio of fungicide-treated pots vs. fungicide-untreated pots in (e) *C. fissa* soil or (f) conspecific soil. Log odds ratios above 0 (the dashed black line) indicate that positive effects and log odds ratios are not significantly different from 0 indicate no effect. Details of the odds ratio regression models fitted are given within each panel, *P*-values of likelihood ratio tests check for significant departure from no relationship with phylogenetic distance. Error bars represent standard errors.

We also found that the survival rates of *C. fissa* and closely related seedlings were significantly lower when seedlings were planted in *C. fissa* soil at high density than at low density, but no significant differences were found for the four more distantly related species (Fig. 4, top panel). Pots sprayed with the fungicide Captan WP for all eight species showed no significant differences in seedling survival between low and high seedling densities (Fig. 4a), but the density effect was significantly negatively correlated with phylogenetic distance in the control pots (Fig. 4b).

DISCUSSION

The field census and the shade-house experiment both suggest that negative effects of tree species on seedling survival are strongest for closely related species, and the shade-house experiment provides evidence that these effects are due to soil-borne pathogenic fungi associated with these trees. These results confirm the conventional wisdom that host-specific interactions between plants and pathogens are often phylogenetically conserved (Farrell 2001). Our study not only improves our understanding of how the Janzen–Connell hypothesis affects species coexistence but may also have implications in disease control in intercropping agricultural systems. If the patterns we observed are general, and extend to agricultural systems, crops should be planted in a design such that the phylogenetic distance between neighbouring individuals is maximised.

Our results indicate that pathogenic fungi in the soil, harvested beneath adult conspecific trees, are important determinants of seedling survival for all species, and that *C. fissa* soil has a negative influence on closely related seedlings, but this effect declines with increasing phylogenetic distance. Thus, these results demonstrate a phylogenetic Janzen–Connell effect that disappears when treating soil with fungicide. Hence, it is reasonable to assume that evolutionary distance is a proxy for the functional response to pathogen attack.

Pathogens have been shown to play a key role in maintaining and structuring diversity in a considerable number of natural and artificial plant systems (reviewed by Gilbert 2002). However, many studies suggest that pathogens and herbivores are often polyphagous rather than monophagous, and many of them may attack host species that are evolutionarily related (e.g. Novotny *et al.* 2002; Agrawal & Fishbein 2006; Gilbert & Webb 2007; Bagchi *et al.* 2010). The results from our study are consistent with this 'phylodiversity-dependent' seedling survival hypothesis (Mangan *et al.* 2010). It is clear that the survival rate significantly decreased when a seedling germinated in the proximity to conspecific or congeneric adult trees. More distantly related adult trees from different genera had no such negative effects and even allowed significantly higher seedling survival in the investigated tree communities compared with closely related adults. Herein, we noticed a gradual increase in seedling survival with phylogenetic distance, and not a sudden jump (Fig. 3). This implies that fungi have probably not co-speciated, but rather, if anything, co-evolved with their preferred host.

Density dependence was only manifest in the fungicide-untreated pots, and the strength of the effect decreased with increasing phylogenetic distance (Fig. 4). These results further confirmed the phylogenetic Janzen–Connell effect caused by soil pathogens. Seedling survival was not significantly different between high and low densities in fungicide-treated soil, indicating that the seedlings did not compete with each other for resources even at considerably high density ($\approx 240 \text{ m}^{-2}$).

The rate of spread of a soil-borne pathogen generally increases with host density (Burdon & Chilvers 1982). As pathogens are able to infect several phylogenetically related species, host density is a function of the number of closely related species in a plant community. Thus, species are expected to experience stronger negative effects at higher densities of phylogenetically closely related species (e.g. species among a large genus), because pathogens
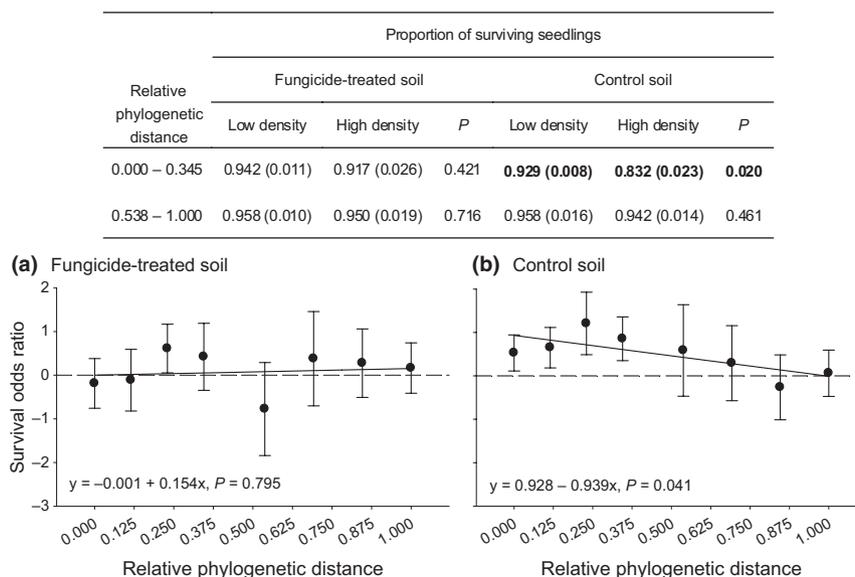
| Relative phylogenetic distance | Proportion of surviving seedlings | | | | | |
| | Fungicide-treated soil | | | Control soil | | |
	Low density	High density	P	Low density	High density	P
0.000 – 0.345	0.942 (0.011)	0.917 (0.026)	0.421	**0.929 (0.008)**	**0.832 (0.023)**	**0.020**
0.538 – 1.000	0.958 (0.010)	0.950 (0.019)	0.716	0.958 (0.016)	0.942 (0.014)	0.461

(a) Fungicide-treated soil
y = −0.001 + 0.154x, P = 0.795

(b) Control soil
y = 0.928 − 0.939x, P = 0.041

Figure 4 Survival of tree seedlings while planted in the *C. fissa* soil at low and high seedling density (4 or 12 seedlings were planted into each pot) in the fungal exclusion experiment performed in a shade-house at the field site. Survival odds ratio between low-density pots vs. high-density pots in (a) fungicide-treated soil and (b) control soil. Log odds ratios above 0 (the dashed line) indicate positive effects of density and log odds ratios not significantly different from 0 indicate no effect. Details of the odds ratio models fitted are given within each panel, P-values of likelihood ratio tests check for significant departure from no relationship with phylogenetic distance. Error bars represent standard errors.

infecting them will spread faster when their host plants are more common. A study determining host specificity of herbivorous insects at a regional scale also suggests that larger genera are attacked by more specialist herbivores than smaller genera (Novotny *et al.* 2002). In our study area, *Cryptocarya concinna* (Lauraceae) and *Castanopsis fabri* (Fagaceae) are the two most dominant species (contributing 11.57 and 5.38% of individuals in our plots), and so it is likely that these two species are attacked by a high number of natural enemies. However, due to the phylogenetic distance between *Cryptocarya concinna* and *Castanopsis fabri* (the two species with largest phylogenetic distance in our shade-house experiment), they are unlikely to be infected by common pathogens. This might explain the coexistence of these two species as codominants in the same community.

During the past decade, an increasing number of studies have analysed the phylogenetic structure of communities to provide insights into the ecological processes of community assembly, organisation and species co-occurrence (reviewed by Cavender-Bares *et al.* 2009). Our data demonstrate that soil-borne fungal pathogens affected seedling survival of phylogenetically related host species as suggested by Webb *et al.* (2006). Their predictions on negative density-dependent interactions among relatives mediated by pathogens or herbivores are consistent with our observation that the investigated tree community was more phylogenetically overdispersed than expected by chance (Webb *et al.* 2002).

In summary, we suggest that the phylogenetic and spatial distance between trees can influence the interaction between trees and pathogenic fungi, and hence promote coexistence of different species in a natural community. This is in line with an increasing awareness of the importance of evolutionary relationships and spatial neighbourhood effects in forests to make the theories of species coexistence more comprehensive (Novotny *et al.* 2002; Webb *et al.* 2006; Gilbert & Webb 2007; Bagchi *et al.* 2010; Metz *et al.* 2010; Ness *et al.* 2011). Our data suggest that the strength of negative effects by neighbouring trees varies considerably depending on the phylogenetic distance between neighbours. Future research will be required to analyse the ecological significance of underground interactions between trees and specific fungal isolates in detail.

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SUPPORTING INFORMATION

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

Figure S1 Details of seedling quadrat network design.
Figure S2 Effects of different evolutionarily related adult trees on seedling survival within a radius of 30 m, 20 m and 10 m in the field survey.
Figure S3 Effects of soil-borne fungi on seedling survival when combining the low and high densities and excluding the zero-distance treatment.
Table S1 Contents of nutrients and soil respiration rates in the fungicide-treated and control soil of each species pots at the end of the fungal exclusion experiment.

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