

Leaf diseases drive the Janzen–Connell mechanism regardless of light conditions: a 3-year field study

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Abstract In forests, negative density/distance-dependent seedling mortality (NDD) caused by natural enemies plays a key role in maintaining species diversity [Janzen–Connell (J–C) model]. However, the relative importance of natural enemies in mediating NDD under heterogeneous light conditions has remained unclear. We examined the relative importance of pathogens (i.e., soil pathogens, leaf diseases) on seedling performance in forest understories (FUs) and gaps (gaps) during a 3-year period (results of first year of our study have been previously reported). For the hardwood, *Prunus grayana*, we investigated seedling mortality, morbidity agents, growth, and root infection by arbuscular mycorrhizal fungi (AMF) beneath conspecific and heterospecific adults in FUs and gaps. Seedling mortality was higher beneath conspecific than heterospecific adults throughout 3 years at both sites, mainly due to continuous leaf disease (i.e., angular leaf spot), whereas damping-off diseases caused mortality only in the first year. Beneath each adult, seedling mortality was higher in FUs than in gaps until second year, but it did not differ between two habitat types in the third year, because leaf diseases caused severe damage even in gaps. Seedling mass was significantly lower beneath conspecific adults. AMF infection of

seedlings was also lower beneath conspecific adults, while it was higher in gaps than in FUs beneath both adults. This study demonstrates that the J–C model in a hardwood tree, *P. grayana* is mainly driven by high NDD seedling mortality caused by airborne leaf diseases, which continuously attack seedlings in a NDD manner regardless of environmental light conditions.

Keywords Angular leaf spot · Arbuscular mycorrhizal fungi · Gaps · Soil pathogen · Species diversity

Introduction

The Janzen–Connell (J–C) model is commonly used to explain species diversity in forest communities (Janzen 1970; Connell 1971). Specially, it proposed that negative density- and/or distance-dependency (NDD) in juvenile mortality caused by species-specific natural enemies was a key factor in maintaining high tree diversity. There is an increasing body of evidence to support NDD seedling mortality in both tropical and temperate forests (e.g., Harms et al. 2000; Packer and Clay 2000; Hyatt et al. 2003; Kulmatiski et al. 2008; Mangan et al. 2010; Konno et al. 2011; McCarthy-Neumann and Ibanez 2013; Bagchi et al. 2014; Comita et al. 2014), but few studies have considered all causes of NDD simultaneously.

Numerous studies in tropical forests have revealed that soil pathogens and insect herbivores are important agents in determining NDD (Wright 2002; Terborgh 2012). Fungicide experiments, which have been the major tool employed to explore NDD seedling mortality in both tropical and temperate forests, have revealed the important role of soil-borne pathogens in determining NDD (e.g., Bell et al. 2006; Kotanen 2007). Furthermore, a recent study on pesticide

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application in a tropical forest demonstrated that soil pathogens were more important in determining NDD than insect herbivores, which affected only the abundance of tree species (Bagchi et al. 2014). These studies on pesticide application assumed that NDD seedling mortality was caused primarily by soil-borne pathogens (e.g., fungi, Oomycota), which cause damping-off diseases in young seedlings. Recent field studies in temperate forests, however, reported that seedling mortality caused by soil-borne damping-off diseases occurred primarily during the early growing season; thereafter, leaf diseases caused mortality in seedlings in an NDD manner (Seiwa et al. 2008; Yamazaki et al. 2009; Bayandala et al. 2016). Furthermore, a reciprocal seed sowing experiment revealed that leaf diseases caused more severe mortality compared with the damping-off diseases in an NDD manner in the current-year seedlings of two hardwood species, *Cornus controversa* and *Prunus grayana* (Bayandala et al. 2016). The synergistic negative effects of soil pathogens and leaf diseases may reinforce the J–C mechanism through significantly stronger NDD compared with the single effects of soil-borne pathogens. However, the duration of and the extent to which leaf diseases continue to affect the NDD and subsequent J–C mechanisms remain to be studied.

It has been thought that the damage caused by soil-borne damping-off diseases may be greater during the early stage of seedling growth and decrease with increasing plant maturity, because the infection of large and old plants is often limited to the fine roots (Sahashi et al. 1995; Martin and Loper 1999; but see Packer and Clay 2003). In contrast, leaf diseases would be expected to significantly reduce plant performance throughout the juvenile stage irrespective of plant age (see Seiwa et al. 2008), because each year the leaves of adult trees infected by air-borne pathogens generally fall on juveniles in the vicinity of the adults (Konno and Seiwa, unpublished data). It remains unexplored how and the extent to which relative influence of damping-off pathogens and leaf diseases on seedling mortality changes with the growth stage of juveniles. As the cumulative impact of NDD effects likely increases with the amount of time that an individual is exposed to natural enemies, longer-term studies would be more likely than shorter ones to detect significant effects of NDD on survival (see Terborgh 2012; Comita et al. 2014). In this study, we explored the relative importance of the two types of pathogens (i.e., soil-borne damping-off diseases and air-borne leaf diseases) in determining NDD seedling mortality until the end of the third growing season.

Although abiotic environments are remarkably heterogeneous along the gap—understory continuum in forest ecosystems (Denslow 1987; Seiwa 1998), the J–C hypothesis has been intensively tested in shaded forest understories (FUs; Packer and Clay 2000; Tomita et al. 2002; Mangan et al. 2010; Alvarez-Loayza and Terborgh 2011), but it has

rarely been studied in light-abundant gaps (gaps). Studies assessing the J–C hypothesis under both types of light conditions have demonstrated stronger NDD caused by soil pathogens under shaded than under high-light conditions (Augspurger 1983, 1984; O’Hanlon-Manners and Kotanen 2004; McCarthy-Neumann and Ibanez 2013). In contrast, in a reciprocal seed-sowing experiment, leaf diseases attacked the current-year seedlings in an NDD manner not only in FUs but also in gaps (Bayandala et al. 2016); however, the absolute survival rate in gaps was relatively high (55.7%), even beneath conspecific adults at the end of the first growing season. If such high survivorship continues after this stage, is it not applicable at high light levels. Multi-year studies, beyond first year survival are necessary to evaluate whether the J–C model is valid in gaps.

Exposed light conditions often enhance the infection rate of mycorrhizal fungi, which have positive effects on seedling performance near adults (Dickie et al. 2002; Hood et al. 2004; Nara and Hogetsu 2004). If the occurrence of the negative and positive effects of microbial communities on seedling performance is largely influenced by the environmental light conditions, it would be useful to study the mechanism by which and the extent to which the relative negative effects of accumulating antagonists (e.g., damping-off pathogens and leaf diseases) versus the relative positive effects of accumulating mutualists (e.g., mycorrhizae) change according to environmental light conditions.

In this study, we explore whether the J–C model is applicable in gaps using the reciprocal seed-sowing experiment described in Bayandala et al. (2016). To evaluate the ontogenetic changes in the relative importance of damping-off diseases and leaf diseases in determining seedling mortality of *P. grayana* seedlings beneath conspecific vs. heterospecific (i.e., *C. controversa*) adults, we measured the causes of mortality in the second year in addition to in the first growing season reported in Bayandala et al. (2016). Furthermore, we investigated the infection of mycorrhizal fungi and seedling mass in the third growing season to evaluate the effects of mutualists on seedling performance beneath conspecific vs. heterospecific adults.

Materials and methods

Species and forest studied

This study was conducted in a deciduous broad-leaved forest in the experimental forest of the Field Science Centre (FSC) of Tohoku University in northeastern Japan (38°48’N, 140°44’E, altitude 500–610 m). Mean monthly temperatures ranged from –2.4 °C (January) to 23.7 °C (July) during the study years (2011–2013). Mean annual temperature and rainfall were 10.3 °C and 1616 mm, respectively.

During winter (December–April), the maximum snow depth is ~1.0 m. Trees in the study area have re-established after the clear-cutting of fuel forests or the abandonment of pasture 40–60 years ago, and the area has not been influenced by human activity for at least the past 40 years. The canopy layer is dominated by *Quercus mongolica* var. *grosseserrata* and *Fagus crenata* as mosaic patches, and the total basal area of the two species is ~48% (Terabaru et al. 2004). Other major canopy trees are *Castanea crenata*, *Aesculus turbinata*, *Carpinus laxifolia*, *Acer mono*, *Quercus serrata*, and *Acer palmatum* var. *matsumurae*. The relative basal area of the two study species is less than 0.97%.

The tree species, *Prunus grayana* and *Cornus controversa* (hereafter referred to by their genus names), are deciduous broad-leaf monoecious trees, common in cool-temperate regions of Japan. In both species, flowering occurs from May to June, and fruits ripen from September to October. *Prunus* seeds are usually dispersed by birds, although most are disseminated beneath the canopy (Seiwa et al. 2008), and the seeds germinate the following spring. Large fruit crops occur every 2–3 years, and dense seedling mats often form the following year (Seiwa et al. 2008). In shaded forest understories, *Prunus* juveniles persist for more than 20 years with relatively short (maximum height = 3 m) monolayer canopies with shrubby multi-stems (Hara et al. 1991; Seiwa et al. 2008). After gap formation, they rapidly grow into the canopy layer and start to reproduce (Hara et al. 1991).

Seed-sowing experiment

In a 50-ha area of the FSC, we selected 14 *Cornus* and 13 *Prunus* adult trees located at least 25 m from the nearest *Cornus* or *Prunus* adult (Bayandala et al. 2016). Two light-level treatments were designed: leaving the forest understory intact (FU) and opening the canopy near the focal tree (gap). For the gap-treatment, we randomly selected seven focal trees of each species and created a 7-m-radius gap around the tree bole by felling all trees and shrubs. The total number of Gap-treated focal adults was seven for each species, and the total number of FU-treated focal adults was six and seven for *Prunus* and *Cornus*, respectively. Three quadrats (35 × 45 cm) were randomly established within a 5-m range of each focal adult bole. In each of the three quadrats, 70 *Prunus* seeds were sown. The total number of *Prunus* seeds sown was 5460. To avoid severe seed predation by small mammals during the winter, the quadrats were covered with 0.2 × 0.2-cm mesh nets, whose edge was buried to a depth of 10 cm. The height of the nets was 10 cm. The nets were removed when seedlings began to emerge the following spring. Canopy openness (%) was lower in FUs than in gaps beneath the *Cornus* (FUs: 13.29 ± 0.25; gaps: 21.73 ± 0.73) and *Prunus* adults (FUs: 3.73 ± 0.92; gaps: 24.22 ± 0.83), whereas few differences

were observed in soil water, pH, or concentrations of carbon (C), phosphorus (P), and nitrogen (N); however, the soil water content was lower in FUs than in gaps beneath *Cornus* adults (Table S1). Further details of the experimental design are provided in Bayandala et al. (2016).

Seedling survival and causes of mortality

All newly emerging *Prunus* seedlings in the two quadrats were tagged and monitored for survivorship until the end of the third growing season under each focal tree. The number of seedlings observed was provided in Table S2. All sources of seedling mortality were investigated during the first (2011; Bayandala et al. 2016) and second (2012) growing seasons (a total of five times) for all seedlings. The causes of mortality were classified as follows: (1) damping-off disease (initial necrosis near the soil level, leaf wilt, and finally, dislodging from the weakened stem); (2) leaf disease (for further identification, see below); (3) invertebrate herbivores (seedling death caused by predation of hypocotyls, >80% of leaf area and/or roots); (4) vertebrate herbivores [severing of main stems by mammals such as wood mice (*Apodemus speciosus*, *A. argenteus*), rabbits, and Japanese serows; we distinguished symptoms from vertebrates from those of pathogens and invertebrates based on the traces from mammals, such as the shape of teeth marks and cut edges, animal droppings and soil that had been turned by mammals]; (5) physical damage (uprooting, drying, fallen branches, and snow pressure); and (6) unknown.

Foliar pathogens were identified as follows: beneath *Prunus* adults, the death of *Prunus* seedlings was characterized by early shedding of leaves showing necrotic spots (5 mm in radius) with observable conidia and conidiophores characteristic of *Phaeoisariopsis*. Using these symptoms, we identified the pathogen as an angular leaf spot caused by *Phaeoisariopsis prunigrayanae* Sawada (Seiwa et al. 2008; Bayandala et al. 2016). Beneath *Cornus* adults, the most common form of seedling morbidity was characterized by early shedding of leaves, demonstrating zonate necrosis with discoid propagules on both the upper and lower surfaces caused by a pathogen that possessed conidia and conidiophores characteristic of *Haradamyces* (Masuya et al. 2009). We identified the pathogen as zonate leaf blight disease caused by *Haradamyces foliicola* (Masuya et al. 2009; Bayandala et al. 2016).

Seedling mass and root colonization by arbuscular mycorrhizal fungi

To estimate colonization of arbuscular mycorrhizal fungi (AMF) and seedling mass, 2-year-old *Prunus* seedlings were harvested (a total of 54 and 60 in FUs and gaps, respectively, beneath *Cornus* adults; and a total of 42 and

47 in FUs and gaps, respectively, beneath *Prunus* adults) on October 29, 2013. Seedlings were carefully removed from the ground by hand and, using a cutlass, washed with water; a total of 0.03 g (wet mass) of roots were then cut to estimate AMF colonization. The seedlings were dried at 70 °C for 60 h before weighing.

The roots were rinsed with tap water and cleaned by heating in 10% KOH at 90 °C for at least 1–2 h. The cleaned roots were rinsed with distilled water and bleached in 0.1% H₂O₂ for 15 min. The bleached roots were then rinsed with tap water and fixed in 2% HCl. The fixed roots were stained with Trypan blue and stored in lactoglycerol (lactic acid 875 mL, glycerin 63 mL, made up to a volume of 1 L with distilled water; Phillips and Hayman 1970; Gehring and Connell 2006; Fukasawa 2012). Root samples (1–3-cm segments of roots from each plant) were mounted on slides and viewed under a compound microscope at 200× magnification (McGonigle et al. 1990) to determine the percentage of the root length colonized by fungal structures. The presence of arbuscular mycorrhizal fungal arbuscules, vesicles, and coils was scored at 150 randomly selected intersections of root and reticle line per plant.

Statistical analysis

Since the data about seedling survival a binomial, the binomial logistic analysis was performed as follows:

$$\ln \frac{p_t}{1 - p_t} = a_0 + a_1 \text{ adult species} + a_2 \text{ light level} + |\text{adult}\$quadrate, \quad (1)$$

where a_i ($i = 0-2$) is coefficient and p_t indicates seedling survival (alive = 1 and dead = 0) at year t (2011–2013), respectively. The terminal term indicates a random effect, and *quadrate* is nested within *adult*. *Adult species* and *light level* are categorical variables to explain the difference in the adult tree species above the quadrats (“heterospecific” or “conspecific”) and the light condition [“forest understoreys (FUs)” or “gaps”]. Logit-link function was used, since the binomial distribution was assumed for the model.

To evaluate the biotic environmental condition that causes the seedling mortality [leaf disease (LD), invertebrate herbivores (IH), and vertebrate herbivores (VH)], the multinomial logistic analysis was performed as follows:

$$\ln \frac{P_{\text{Cause}}}{p_{\text{IN}}} = b_0 + b_1 \text{ adult species} + b_2 \text{ light level}, \quad (2)$$

where b_i ($i = 0-2$) is coefficient. P_{Cause} indicates seedling mortality caused by LD, IH, and VH, whereas, p_{IN} is the mortality of survived seedlings. In this model, the mortality of survived seedlings is the baseline-category, that is, the relative contribution of both adult species and light level to the seedling mortality against with the survived seedlings

(IN). Here, dumping-off was eliminated from the analysis because of few data ($n = 2$). Multinomial distribution was assumed for the model.

Effects of adult species and light level on the infection of AMF and seedling mass were evaluated by the linear mixed model (LMM) as follows:

$$\text{Infection of AMF or Seedling mass} = c_0 + c_1 \text{ adult species} + c_2 \text{ light level} + |\text{adult}\$quadrate, \quad (3)$$

where c_i ($i = 0-2$) is coefficient. The terminal term indicates a random effect, and *quadrate* is nested within *adult*. Gaussian distribution was assumed for the model. Akaike’s information criterion (AIC) was used to select the best-fit model (Anderson and Burnham 2001). Fixed variables were successively deleted from the full model, including all variables mentioned above. We used R ver. 3.3.1 (The R Foundation for Statistical Computing 2016) with *glmer* function in the package *lme4* for the binomial logistic model and LMM, and with *vglm* function in the package VGAM for the multinomial logistic model. Random effects were omitted if their inclusion did not improve the fit of the model. We used *glm*, instead of *glmer*, if random effects were not included.

Results

Seedling survival

The seedling survival of *Prunus* was greater beneath *Cornus* than beneath *Prunus* adults across all sampling times (Fig. 1), and in fact, the model with adult species was

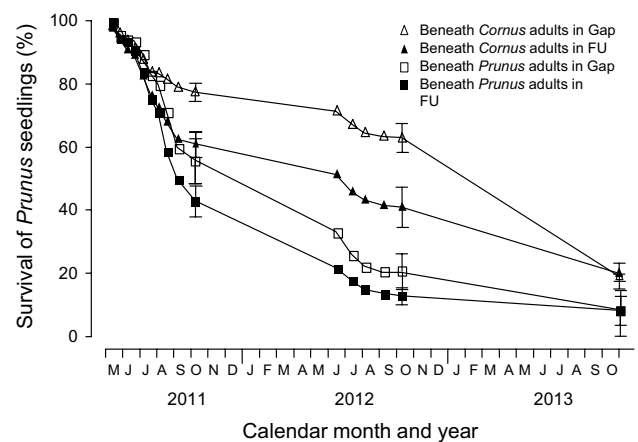


Fig. 1 Seedling survival of *Prunus grayana* over a 3-year period following seedling emergence in forest understoreys (FUs) and gaps (gaps) beneath heterospecific (i.e., *Cornus controversa*) and conspecific (*P. grayana*) adults

Table 1 Effects of heterospecific vs. conspecific (i.e., beneath *Cornus controversa* adults vs. beneath *Prunus grayana* adults), and forest understorey (FUs) vs. gaps on seedling survival of *P. grayana* using binomial logistic analysis

Seedling age (observed year)	Intercept	z	Adult species		Light level	
			Heterospecific vs. conspecific		FUs vs. gaps	
			Coeff.	z	Coeff.	z
0 year (2011)	0.529	3.409	−0.995	−5.622	0.727	4.120
1 year (2012)	−0.314	−1.642	−1.798	−8.097	0.612	2.760
2 years (2013)	−1.403	−19.192	−1.098	−8.855	–	–

Heterospecific and FUs are the reference categories (=0). *Glm* function was used instead of *glmer* function in the analysis of 2-year-old seedlings (2013)

selected in the binomial logistic analysis for consecutive 3 years (Table 1).

Beneath the adults of both species, seedlings survival was generally higher in gaps than in FUs until the end of the second growing season (Table 1; Fig. 1). But light level was excluded from the model for 2013 (Table 1; Fig. 1), immediately means that the effect of light condition on the seedling survival vanished until the end of the third growing season (Fig. 1). It is noted that the model with the intercept only is the case of ‘heterospecific’ with ‘FUs’; i.e., the seedling survival beneath *Cornus* in the forest understorey. The size of intercept decreased year-to-year, immediately corresponds to the decrease of seedling survival beneath *Cornus* in the forest understorey.

Causes of seedling mortality

For 1-year-old *Prunus* seedlings, the most morbidity agent was leaf diseases, followed by vertebrate herbivores (mainly wood mice), whereas no seedlings were killed by damping-off diseases in either FUs or gaps beneath *Cornus* and *Prunus* adults (Fig. 2). For the relative probability of morbidity caused by leaf diseases, $|z|$ value of adult species (+) and light level (−) was >2 indicating that the seedling mortality was higher beneath *Prunus* than beneath *Cornus* (Table 2) and was higher in FUs and in gaps (Table 2). Seedling mortality caused by vertebrate herbivores was also higher beneath *Prunus* than *Cornus* adults ($z = 7.109$), but it did not differ between the light levels (Table 2).

Similar to Table 1, the model with the intercept only is the case of ‘heterospecific’ with ‘FUs’. The result suggests that the seedling mortality beneath *Cornus* in the forest understorey decreased in the order of ‘leaf disease’, ‘invertebrate’, and ‘vertebrate’.

The most prevalent leaf diseases beneath *Cornus* and *Prunus* adults were zonate leaf blight and angular leaf spot caused by the airborne pathogenic fungi *Haradamyces foliicola* and *Phaeoisariopsis pruni-grayanae*, respectively. Beneath *Cornus* adults, other leaf diseases were also observed, but their occurrence relative to zonate leaf blight and angular leaf spot was negligible.

Infection of AMF and seedling mass

GLM analysis revealed that both adult species and light level contributed to the percent infection of AMF and seedling mass (Table 3). The coefficient of adult species was negative indicating that the percent infection of AMF in *Prunus* seedlings was higher beneath *Cornus* than beneath *Prunus* (Fig. 3a). On the other hand, the coefficient of light level was positive indicating that the percent infection of AMF in *Prunus* seedlings was higher in gaps than in FUs.

Similar tendency was observed for the seedling mass, particularly in gaps (Fig. 3b). The seedling dry mass increased with the increasing percentage colonization of AMF beneath both adult species, but the increasing rate was very low beneath *Prunus* adults (Fig. S1).

Discussion

The experimental study of gaps in a temperate forest in northern Japan clearly demonstrated that the J–C hypothesis was valid even in light-abundant gaps (gaps), in addition to shaded forest understoreys (FUs), in a hardwood species, *Prunus grayana*. Although this seed-sowing experiment already demonstrated that *Prunus* seedlings died in a negative distance-dependent (NDD) manner in the first growing season even in gaps, the absolute mortality was remarkably high (55.7%; Bayandala et al. 2016), suggesting that the J–C model would be applicable in the gaps (Nathan and Casagrandi 2004). As expected, a 3-year period of observations demonstrated that NDD seedling mortality was continuously observed not only in FUs but also in gaps, with minor differences in the strength of NDD between the two habitat types (i.e., gaps vs. FUs). This was mainly due to the severe attack of a leaf disease, angular leaf spot, which occurred irrespective of the light conditions beneath conspecific adults.

Beneath *Prunus* adults, the most prevalent leaf disease, angular leaf spot, caused more severe leaf damage and earlier leaf shedding in the *Prunus* seedlings relative to that occurring beneath heterospecific adults (*Cornus*) in both

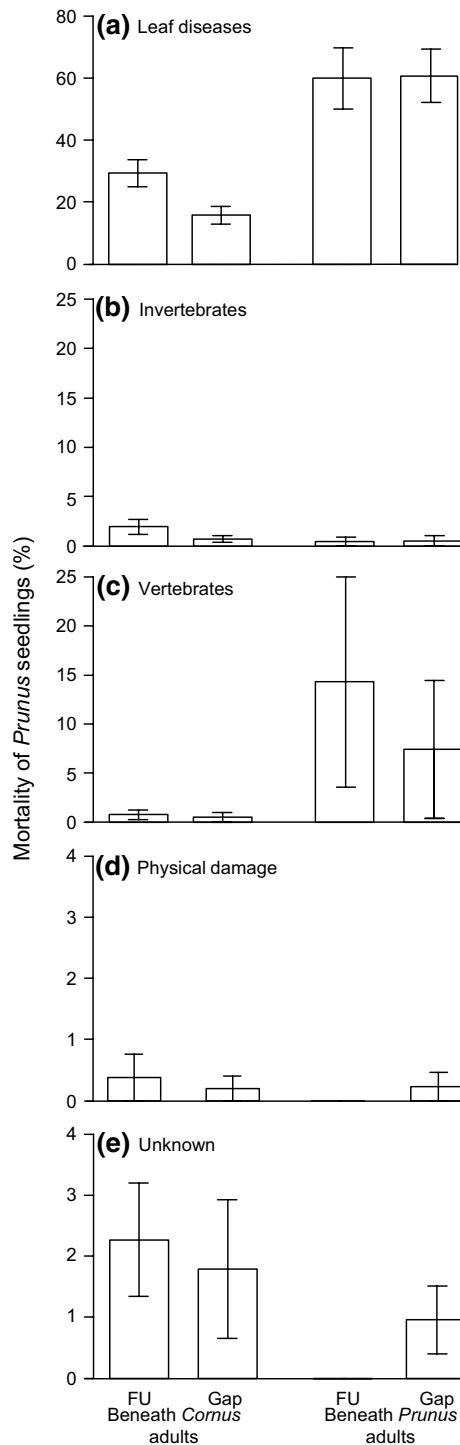


Fig. 2 Mortality of 1-year-old *Prunus grayana* seedlings caused by **a** leaf diseases, **b** invertebrates, **c** vertebrates, and **d** physical damage in forest understories (FUs) and gaps (gaps) beneath heterospecific (*Cornus controversa*) and conspecific (*P. grayana*) adults. Values are mean \pm SE ($n = 272, 420, 330, 478$, respectively)

FUs and gaps in the second growing season. This was similar to that already observed in the first growing season (Bayandala et al. 2016), suggesting a continuous strong

NDD attack by the leaf disease (Fig. S2). Furthermore, our previous study found host preference of the angular leaf spot, which caused higher seedling mortality in *Prunus* than in *Cornus* seedlings, beneath *Prunus* adults (Bayandala et al. 2016). The leaves infected by angular leaf spot were shed every autumn within a 10- to 15-m radius around the boles of *Prunus* adults (Yamazaki et al. unpublished data). Hence, *Prunus* seedlings beneath the adults suffered severe damage annually. Thereafter, following the J–C model, higher seedling mortality would be expected to also be observed in conspecific seedlings throughout the juvenile stage.

Furthermore, our 3-year observation clearly revealed that the leaf disease, angular leaf spot, continuously attacked the conspecific seedlings throughout the juvenile stage irrespective of light conditions, whereas soil-borne damping-off diseases attacked only the current-year seedlings, especially in shaded FUs (Fig. S2; Bayandala et al. 2016). Previous studies have also reported that soil-borne pathogens were less effective under light-abundant conditions compared with shaded forest understories (Augspurger 1983; Hood et al. 2004). In this study, we clearly revealed that the airborne leaf diseases play a more important role compared with that of soil pathogens in facilitating NDD seedling mortality and subsequent the J–C mechanism even in light-abundant gaps.

Although most of the NDD studies to date indicate that NDD is most important in the earlier stages of plant growth, several studies have reported NDD patterns in the recruitment, growth, and survival in the sapling and adult stages (Peters 2003; Stoll and Newbery 2005; Piao et al. 2013; but see Zhu et al. 2015). This study also suggests that the effects of negative-distance dependency (NDD) on plant performance likely persist beyond the seedling stage, when the plants are strongly affected by highly virulent leaf diseases. This suggests that researchers must be mindful that different enemies (microbial, invertebrate, vertebrate) may be important in different systems and that studies that characterize effects over greater periods of time are likely the most relevant for interpreting the factors that regulate forest composition and diversity.

Beneath *Cornus* adults (i.e., “away”), infection rates of AMF in *Prunus* seedlings were higher in gaps than in FUs, indicating that abundant light enhances the occurrence of AMF (Gehring and Connell 2006). However, the large seedling mass in gaps may not be simply caused by the increased infection rate of AMF (Fig. S1), because it is possible that antagonists are simply reducing the *Prunus* seedlings’ performance. Further experimental study such as inoculation experiments in greenhouse should be needed to clarify the effects of AMF on seedling performance.

Recent studies demonstrated that rare species with a small relative abundance showed significantly stronger

Table 2 Results of the multinomial logistic analysis of the effects of heterospecific vs. conspecific (i.e., beneath *Cornus controversa* adults vs. beneath *Prunus grayana* adults) and forest understorey vs. gaps on the mortality of 1-year-old seedlings attribute to different agents (i.e., leaf disease, invertebrate herbivores, vertebrate herbivores and others)

Agents	Intercept	z	Adult species		Light level	
			Heterospecific vs. conspecific		FUs vs. gaps	
			Coeff.	z	Coeff.	z
Leaf disease	-0.942	-8.743	1.839	14.875	-0.568	-4.571
Invertebrates	-3.595	-9.351	-0.176	-0.264	-0.961	-1.668
Vertebrates	-4.983	-10.038	3.366	7.109	0.356	1.149

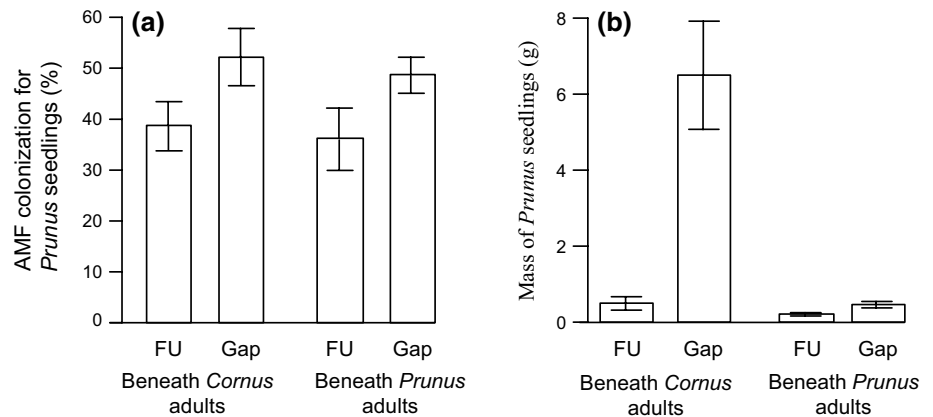
Intact seedlings are baseline of this model. Heterospecific and FUs are the reference categories (=0)

Table 3 Effects of heterospecific vs. conspecific (i.e., beneath *Cornus controversa* adults vs. beneath *Prunus grayana* adults) and forest understorey (FUs) vs. gaps on seedling mass and percent infection of arbuscular mycorrhizal fungi (AMF) in *P. grayana* using generalized linear models (GLM)

	Intercept	t	Adult species		Light level	
			Heterospecific vs. conspecific		FUs vs. gaps	
			Coeff.	t	Coeff.	t
Infection of AMF	42.761	14.997	-10.100	-2.997	13.122	3.910
Seedlings mass	1.711	2.689	-3.007	-4.000	3.138	4.193

Heterospecific and FUs are the reference categories (=0)

Fig. 3 Two-year old *Prunus grayana* seedlings, **a** colonized by arbuscular mycorrhizal fungi (% AMF) and **b** mass (mean ± SE; n = 54, 60, 42, 47) in forest understorey (FUs) and gaps beneath heterospecific (*Cornus controversa*) and conspecific (*P. grayana*) adults



NDD compared with abundant species within a plant community (Comita et al. 2010; Mangan et al. 2010; Anacker et al. 2014). The species in our study, *Prunus*, is a rare species within the community of a temperate old-growth forest (relative basal area = 0.30%; Terabaru et al. 2004). The rare abundance is probably due to the strongly NDD seedling mortality caused by the leaf disease, angular leaf spot even in gaps. This indicates that the strength of NDD is significantly affected by the negative effects of accumulating antagonists (e.g., damping-off diseases and leaf diseases).

In conclusion, our study clearly demonstrated that leaf diseases play a crucial role in mediating the J–C mechanism in gaps, because leaf diseases continuously attack the seedlings in a distance-dependent manner regardless of environmental light conditions.

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Author contribution statement KS conceived and designed the experiments. B and KS performed the experiments. KM analyzed the data. KS and B wrote the manuscript.

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