

Bottom-up effects and conspecific negative density dependence in a subtropical forest

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Abstract

The early growth stage of plants is vital to community diversity and community regeneration. Understandably, it is critical to explore the mechanisms underpinning the spatial and temporal dynamic patterns of seedling survival and growth. The Janzen–Connell hypothesis predicts that conspecific density dependence lowers the survival of conspecific seedlings by attracting specialist natural enemies, promoting the recruitment and performance of heterospecific neighbors. Recent work has underscored how this conspecific negative density dependence may be mediated by mutualists – such as how arbuscular mycorrhizal fungi may mediate the accrual of host-specific pathogens beneath the crown of conspecific. Aboveground mutualist and enemy interactions exist as well, however, and may provide useful insight into density dependence that are as of yet unexplored. Using a long-term seedling demographic dataset in a subtropical forest plot in central China, we found that a mutualist association with ants had significant positive effects on seedling survival, but a negative effect on seedling growth. We also confirmed that conspecific neighborhoods had a significant negative effect on seedling survival, confirming that the Janzen–Connell hypothesis may influence community composition in the subtropical forest. Overall, our findings suggested that ants and conspecific neighborhoods played important but inverse roles on seedling survival and growth, which indicated that ants may mediate conspecific negative density dependence at community level to some extent.

Abstract

The early growth stage of plants is vital to community diversity and community regeneration. Understandably, it is critical to explore the mechanisms underpinning the spatial and temporal dynamic patterns of seedling survival and growth. The Janzen–Connell hypothesis predicts that conspecific density dependence lowers the survival of conspecific seedlings by attracting specialist natural enemies, promoting the recruitment and performance of heterospecific neighbors. Recent work has underscored how this conspecific negative density dependence may be mediated by mutualists – such as how arbuscular mycorrhizal fungi may mediate the accrual of host-specific pathogens beneath the crown of conspecific. Aboveground mutualist and enemy interactions exist as well, however, and may provide useful insight into density dependence that are as of yet unexplored. Using a long-term seedling demographic dataset in a subtropical forest plot in central China, we found that a mutualist association with ants had significant positive effects on seedling survival, but a negative effect on seedling growth. We also confirmed that conspecific neighborhoods had a significant negative effect on seedling survival, confirming that the Janzen-Connell hypothesis may influence community composition in the subtropical forest. Overall, our findings suggested that ants and conspecific neighborhoods played important but inverse roles on seedling survival and growth, which indicated that ants may mediate conspecific negative density dependence at community level to some extent.

Key words: Forest dynamic plot, seedling dynamics, ant presence, negative density dependence, herbivory.

Introduction

Understanding species coexistence in communities is a central goal of ecology. Conspecific negative density dependence (CNDD) suggests that intrinsic limits on conspecific individuals promote diversity and coexistence (Harms *et al.* 2000; Comita *et al.* 2014). A classic example of CNDD is the Janzen-Connell hypothesis, which suggests that the accrual of host-specific seed predators reduces the likely that conspecific seedlings will survival in the area immediately around any given adult host (Janzen 1970; Connell 1971). Many studies have identified CNDD in tropical forests (Comita *et al.* 2010; Hazelwood *et al.* 2021), suggesting that density dependent effects play an essential role in mediating competitive exclusion and maintaining community diversity. A number of recent studies suggest that the Janzen-Connell effect is not confined to tropical forests but also prevalent in subtropical (Chen *et al.* 2010; Zhu *et al.* 2010; Song *et al.* 2021) and temperate forests (Johnson *et al.* 2012; Lamanna *et al.* 2016).

It is obvious, however, that the Janzen-Connell hypothesis is not completely true for all species (Klironomos 2002; Petermann *et al.* 2008; Johnson *et al.* 2017). In the wild, rare species are often associated with high levels of CNDD that prevent them from becoming widespread while common species have commensurately lower estimates of CNDD (Comita *et al.* 2010; Mangan *et al.* 2010). CNDD also likely varies among functional type and ecosystem role. For example, shade-tolerant species (Kobe & Vriesendorp 2011), invasive species (Klironomos 2002) are often less susceptible to CNDD – likely a consequence of evolutionary adaptations designed to quickly colonize

new environments.

It is still unclear, however, what precisely mediates these CNDD effects. Considerable focus has been paid to the influence of belowground mutualists and pathogens on CNDD. For example, recent research has showed that ectomycorrhizal species experience weaker Janzen-Connell effects because ectomycorrhizal fungi protect plants from pathogens damage(Chen *et al.* 2019; Segnitz *et al.* 2020; Jiang *et al.* 2021). These belowground influences may scale to reflect the variation in CNDD we observe in nature. For example, seedlings of common trees may experience a greater benefit from mutualists beneath conspecifics than seedlings of rare tree species – which may be one explanation for why rare tree species are rare(Schroeder *et al.* 2020). These efforts, however, have yet to explore the influence of more obvious plant-animal mutualisms aboveground.

Positive interaction above ground, which is common and crucial component of a number of ecological communities(Bruno *et al.* 2003), plays an important role to alter the dynamics of resource competition, particular in ant-plant interaction, which is one of the most discussed models in the mutualist system(Rico-Gray & Oliveira 2007). In the facultative ant-plant mutualism, plants offer a variety of potential benefits to ants, including extrafloral nectaries (EFNs), honeydew and food body(Heil & McKey 2003). In return, ants act as biotic defense against pathogens and herbivores which may diver CNDD. Ants are abundant and diverse in the terrestrial ecosystems and important natural enemies of a wide range of insect herbivores(Hölldobler & Wilson 1990; Blüthgen *et al.* 2000). Ants can increase plant growth and fitness by suppressing

herbivores pressure(Ito & Higashi 1991; Clark *et al.* 2019). The association between plants and ants is so widespread and successful that ant plants are often ecologically dominant(Parker & Kronauer 2021). It is a logical conclusion that ants may impact the strength of CNDD among species via altering predation pressure.

Although both CNDD and ants are important to plants, the relationship between interaction of ant-plant and CNDD is poorly understood. Here, we conducted a study to detect variation in the CNDD among tree species in a 25-ha Badagongshan subtropical forest dynamic plot in central China. This study focused on seedling performance, because the CNDD is most influential in the seedling stage(Zhu *et al.* 2015).

Materials and Methods

Study site

This study was carried out within a subtropical evergreen and deciduous broad-leaved mixed forest within the Badagongshan (BDGS, 29°46'N, 110°05'E) 25-ha forest dynamics plot (FDP) in central China. This plot is a member of global network of long-term forest dynamic research sites(Condit 1998). The climate is characterized by an annual mean temperature of 11.5°C (0.1-23.3°C) and mean annual precipitation of ~2105 mm. In the BDGS FDP, all individuals with DBH (diameter at breast height, 1.3m)≥ 1 cm have been tagged, mapped, measured and identified at five-year intervals since 2011(Zhang *et al.* 2020). At the first census, the BDGS FDP included more than 187000 individuals which belonged to 232 species, 114 genera and 53 families. The dominate tree species are *Quercus multinervis* and *Fagus lucida*. Other important

species include *Quercus shennongii*, *Quercus serrata*, *Schima parviflora*, *Carpinus viminea*, *Sassafras tzumu*, *Litsea elongata* and *Rhododendron stamineum*(Lu *et al.* 2013).

Predation pressure census

Herbivore pressure on each individual was estimated following (Clark *et al.* 2016). For each individual, herbivore was classified into one of six categories: ‘no damage’, ‘1-20% leaf area damaged’, ‘20-40% leaf area damaged’, ‘40-60% leaf area damaged’, ‘60-80% leaf area damaged’, or ‘80-100% leaf area damaged’. Herbivory that left nothing but remnant petioles was considered 80-100% herbivory. The loss of leaf in each individual was calculated by following formula:

$$A \times \sum_{i=1}^n (C_i \times M_i)$$

where A is the average area of a single leaf for a given tree species. 73 species were selected ten individuals and then scanned ten leaves of each individual and 23 species less than ten individuals. C_i is the number of leaves at different damage levels. If the number of leaves collected from a given individual was greater than 100, we subsampled from three branches from three different directions. M_i is the median proportion of leaf area damaged in the i th damage level.

To estimate herbivory rate, we counted the number of leaves belonging to different categories for each individual in July 2022 when herbivory is widespread. The total area of all leaves was equal to the average area of leaf, multiplied by the number of leaves in the individual. The herbivory rate of each individual was calculated as the loss area of individual divided by the total area of individual. We assessed 2337 individuals

belonging to 96 species for loss of leaf and 2383 individuals belonging to 108 species for herbivory rate eventually.

Seedling census

In 2012, a seed rain census was designed and established, consisting of 135 seed traps arrayed along trails and in the center of the 20×20 m quadrats (Figure S1). Three 1-m² seedling plots were established 2-m away from sides of each seed trap, resulting in 405 1-m² seedling plots. Three adjacent seedling plots and a seed trap together were considered one ‘census station’. The mean distance between stations was 31.03 m. All woody seedlings and recruitments (<1 cm DBH) in the station were tagged, mapped, identified, measured, and censused twice a year (in May and August)(Lu *et al.* 2015). As of 2022, eleven years of seedling data have been collected and the censuses have never been interrupted. A total of 12,313 seedlings of 134 species were tagged from 2012 to 2022. In 2022, there were 2,431 seedlings in May and 2,392 seedlings in August surveyed, belonging to 109 species.

Ant samples collection

During the four seedling censuses of 2021 and 2022, we counted the number of ants on each seedling. We always observed and sampled the ants first, to avoid disturbing them when re-censusing the seedlings. All ant samples were placed in a 5-ml centrifuge tube filled with 95% ethanol (1 vial per seedling) and returned to the lab to be sorted and identified.

For each seedling plot, we sampled for at least 5 minutes(De Queiroz *et al.* 2013; Da Silva *et al.* 2020), in fact the sampling time was often more than 5 minutes if there were

many seedlings or ants in the seedling plot. At the same time, we also sampled the ants patrolling on the seedling outside seedling plot.

Defining associations between seedlings and ants

We summarized the number of ants on each species, and estimated the best fitted distribution using bootstrap method (Figure S2). Comparing the poisson and negative binomial distribution, we found the negative binomial distribution to better match the observations (Figure S3). After confirming the negative binomial distribution, we also compared the cumulative distribution function (CDF) of observed values with our theoretical negative binomial distribution (Figure S4). According to negative binomial distribution, we calculated the mean and standard error of the mean of the samples. If the number of ants on a given species was more than the sum of the mean ant load and $1.96 \times$ standard error, we considered that species to be closely associated with ants (Figure S5).

A total of 26 tree and shrub species were identified as species that are closely correlated to ants (Table S1). We supported these associations with observations of ants foraging on the leaves (Figure S6). We also found direct evidence that ants catch herbivorous beetles, proving their anti-predator protection.

Data analysis

To estimate the effect of ants on herbivory, the leaf loss and herbivory were modeled as a function of ants incidences using linear mixed-effects models (LMM). Plant species were included as random effects to explain the inherent differences.

To evaluate the joint influence of ants tree neighborhood on focal seedlings, we

modeled seedling survival from 2012 to 2021 as a function of ants and neighbors using generalized linear mixed-effects models (GLMM) with a binominal error distribution. We looked to model relative growth rate (RGR) as a function of ants and neighboring plants using LMM. We calculated the RGR by using this formula: $\log(H_{t+\Delta t}/H_t)/\Delta t$, where H is the height of seedling at a certain moment and Δt means the time interval between measurements (Hunt 1982). We added the heterospecific seedling and adult density as covariates simultaneously.

For the seedling survival data, there are 3979 individuals of 26 woody plant species which have close associations with ants and 7896 individuals of 86 plant species unassociated with ants. All other species whose ant-status was unconfirmed were excluded before analysis. All continuous explanatory variables were standardized to compare the relative importance of each variable directly (Gelman & Hill 2006; Jia *et al.* 2020). For the seedling growth data, we did not include any seedling individuals who survived fewer than three months. There were total of 6122 individuals of 110 species who met these criteria. All continuous variables were transformed to be normalized and then standardized. Finally, we explored the relationship between survival and RGR using GLMM. The fixed effect is RGR and random effects were consistent with the survival and growth models.

The crowding index is the basal area of trees in a 20-m radius and the seedling density is the number of seedlings in a 1-m² plot. In order to control spatial autocorrelation, the seedling plot and the station ID were included as nested random effects. We also set the plant species as a random effect to account for inherent differences between plant

species. Lastly, year was also included as a random effect to account for inter-annual variation in survival or growth.

Conspecific and heterospecific adult density were calculated using the sum of the basal area (BA) and divided by the distance from the central of seed traps(Comita & Hubbell 2009):

$$BA = \sum_i^n (BA_i / \text{DISTANCE}_i)$$

All the data analyses were implemented in R v 3.6.3(R Core Team 2020) using the “lme4” package(Bates *et al.* 2015).

Results

Loss of leaves and herbivory rate

In contrast to our expectations, species with close association with ants exhibited a little higher leaf loss than species without ant mutualists ($F = 3.93$, $df = 1$, $p = 0.051$, Fig. 1a). Interestingly, however, there is no significant difference among species in herbivory rate ($F = 1.03$, $df = 1$, $p = 0.31$, Fig. 1b).

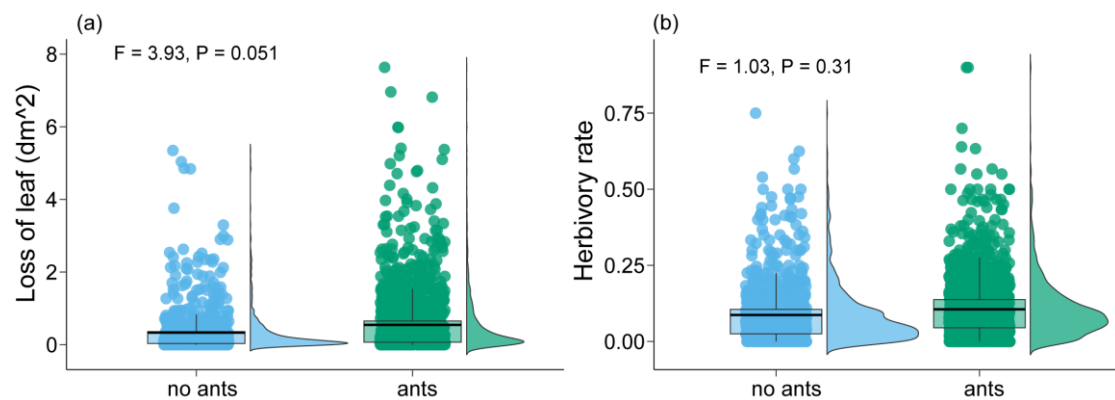


Fig. 1 Variation in leaf loss (a) and herbivore rate (b) among different species. Graphs included boxplot with mean, hinges and whiskers which are the upper and lower non-outlier values within $1.5 \times$ interquartile range, raw data (points) and violin plot grounded

on Kernel density function.

Community level density dependence and ants on seedling survival

A total of 11,717 individuals were analyzed in the GLMM. We found that ants influenced seedling survival significantly (binomial GLMM, ant: $z = 1.977$, $p = 0.048$, Fig. 2a). Consistent with the Janzen-Connell hypothesis, conspecific density had a negative effect on seedling survival. This pattern was especially strong when considering the density of conspecific seedling neighbors, perhaps reflecting increased competition (binomial GLMM, S.con: $z = -4.667$, $p < 0.001$, Fig. 2a). Similarly, the density of heterospecific adults was positively associated with seedling survival (binomial GLMM, A.het: $z = 4.97$, $p < 0.001$, Fig. 2a), also consistent with the Janzen-Connell hypothesis. When we separated the species based on whether they are associated with ants, we found that seedling survival was higher in plants with ant mutualists than in plants without. This was true across a range of neighborhood densities, though the effect of conspecific adult density is not significant (Fig. 2b, c).

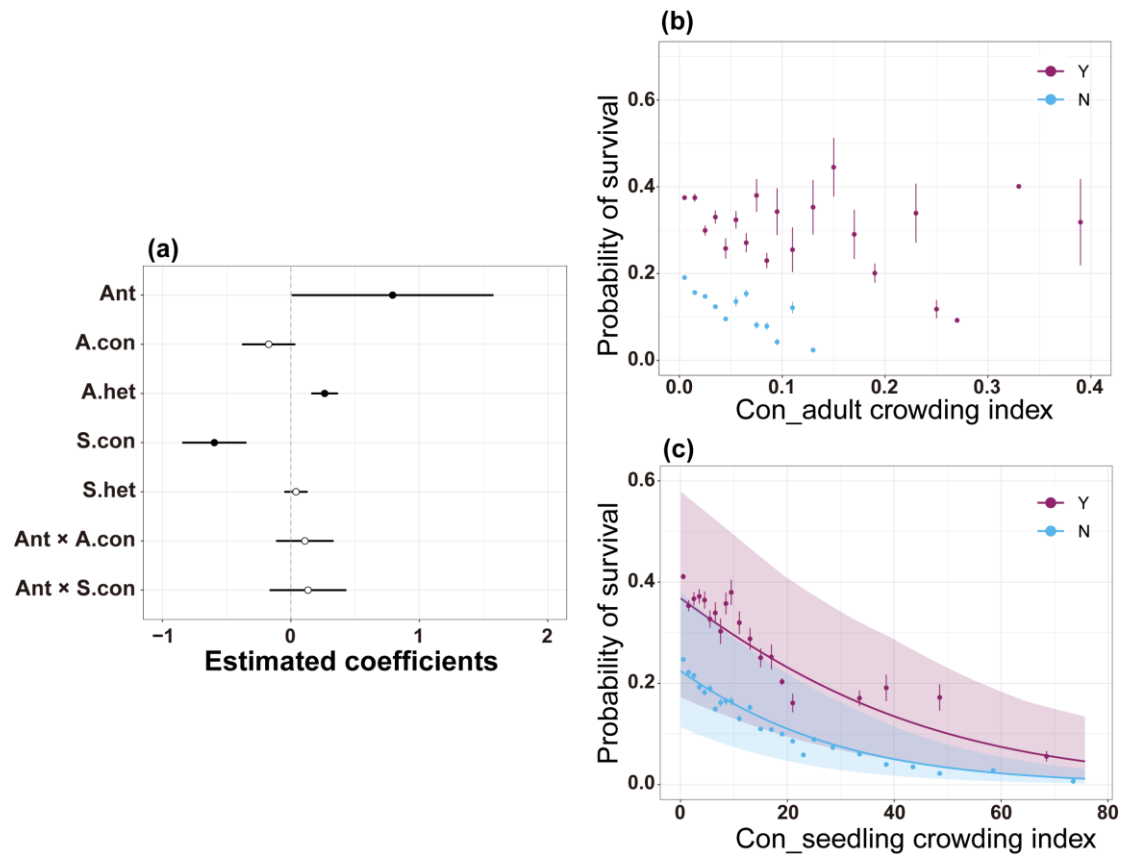


Fig. 2 Effects of ants and neighbors on seedling survival. (a) Parameter estimates from GLMM (Generalized Linear Mixed Model) fitted to seedling survival ($n = 11717$) data from 112 species in the seedling assemblage. Solid points indicated that the parameter estimates are significant. Error bars indicate 95% confidence intervals. (b, c) The relationships between conspecific neighbors and seedling survival under different type of trees (Y: trees with close associations to ants, N: trees with uncorrelation to ants). Lines and shade areas exhibit the GLMM's predictions and 95% confidence intervals. Solid points and error bars indicate the mean and standard error of the observed values, which were computed by putting the model residuals into the predicted values. The means of observed values within bins to facilitate visualization because of too much observed values.

Community level density dependence and ants on seedling RGR

Despite increasing seedling survival, associating with ants had a negative effect on seedling growth (linear mixed-effects model, ant: $t_{81} = -2.195$, $p = 0.031$, Fig. 3a). Similarly, the density of heterospecific adult neighbors reduces seedling growth (LMM: A.het: $t_{159} = -3.165$, $p = 0.002$, Fig. 3a). Unexpectedly, a high proportion of conspecific seedling neighbors is positively associated with seedling growth (LMM, S.con: $t_{3184} = 4.969$, $p < 0.001$, Fig. 3a). When we compared the effects of different species with and without ants along varying neighborhood densities, the seedling growth of species without ants are higher than species with ants even though the effect of conspecific adult density is not significant (Fig. 3b, c).

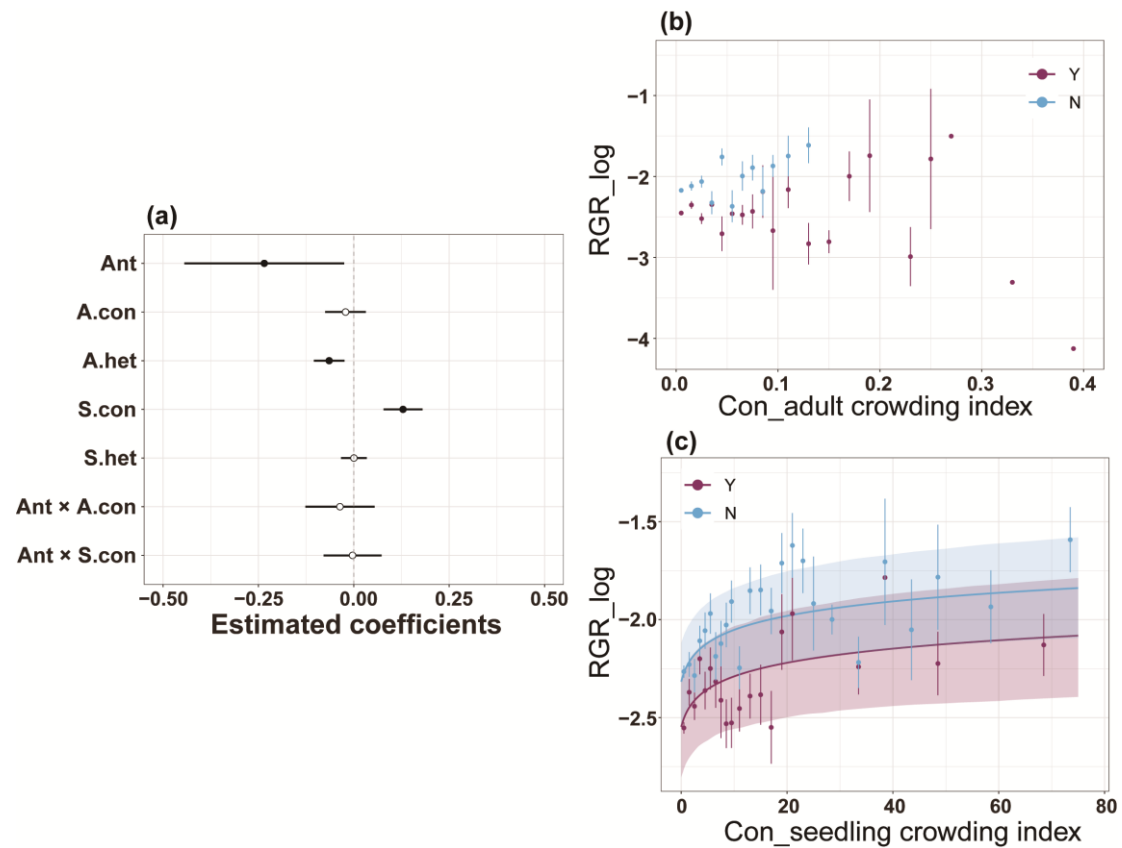


Fig. 3 Effects (odds ratio \pm SE) of ants and neighborhoods on seedling growth. (a) Parameter estimates from LMM (Linear Mixed Model) fitted to seedling growth ($n = 6122$) data from 110 species in the seedling assemblage. Solid points indicated that the

parameter estimates are significant effect and bar indicated 95% confidence intervals.

(b to c) The relationships between conspecific neighbors and RGR under different

type of trees (Y: trees with close associations to ants, N: trees with uncorrelation to ants).

Lines and shade areas exhibit the LMM's predictions and their 95% confidence

intervals. Solid points and error bars indicate the mean and standard error of the

observed values, which were computed by putting the model residuals into the predicted

values. The means of observed values within bins to facilitate visualization because of

too much observed values.

Relationship between seedling survival and growth

GLMM showed that there is a significant negative relationship between survival and

RGR ($z = -22.57, p < 0.001$). The probability of survival is inversely proportional to

RGR (Fig. 4).

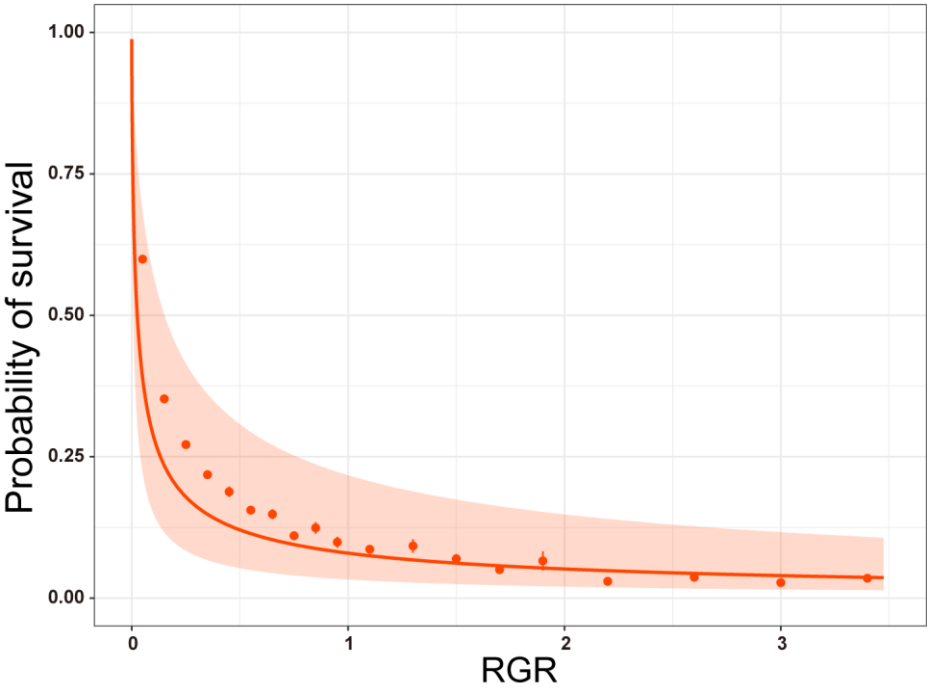


Fig. 4 The relationship between seedling survival and growth. Lines and shade areas

exhibit the GLMM's predictions and 95% confidence intervals. Solid points and error

bars indicate the mean and standard error of the observed values, which were computed by putting the model residuals into the predicted values. The means of observed values within bins to facilitate visualization because of too much observed values.

Discussion

Many factors could influence the strength of CNDD. A handful of studies have demonstrated that temperature, precipitation, topography and altitude may mediate CNDD at the local and regional scale(Xu & Yu 2014; Bachelot *et al.* 2020; Song *et al.* 2020). Recent studies focused on the belowground have identified that specie-specific variation in CNDD may be related to the combined influence of positive and negative soil microbes(Schroeder *et al.* 2020; Jiang *et al.* 2021; Pu *et al.* 2022). For example, ectomycorrhizal fungi have been shown to protect against pathogens, mediating the Janzen-Connell effect and producing overall weaker CNDD(Chen *et al.* 2019; Segnitz *et al.* 2020). Though ants protect plants against herbivores aboveground, they remain unconsidered in these recent explorations of CNDD(Heil *et al.* 2001). Our results showed a mutualist association with ants promotes survival, and that plants closely associated with ants experienced weaker CNDD than plants unassociated with ants (Fig. 2a). That means mutualisms above the ground have positive effects on CNDD.

In the ant-plant mutualism, ants can protect plants against herbivores in return for food rewards(Heil & McKey 2003). A number of studies suggests plants with ants exhibit lower leaf damage and lower rates of herbivory rate(Bruna *et al.* 2004; Bixenmann *et al.* 2011). The associations between plants and ants via extrafloral nectaries and homopteran honeydew are widespread in tropical rainforest(Blüthgen *et*

294 *al.* 2000). However, the proportion of species and individuals with EFNs decreased with
295 latitude(Luo *et al.* 2022). We found many plants in BDGS FDP without EFN or phloem-
296 feeding hemipterans, whereas ants forage occurred in many common species through
297 two years' observation. Ant presence exhibited preference to plants simultaneously. For
298 instance, we never saw ants on the *Ilex pernyi* which is one of common species in BDGS
299 FDP (Table S1). Plants that are extremely attractive to herbivores need to develop
300 mutualism to offset predator pressure. Therefore, it is possible that associations between
301 ants and plants are established by herbivores in subtropical. Plants pay a cost to
302 minimize damage, but they will still be foraged on regularly. Our results showed that
303 plants with ants exhibited higher leaf damage (Fig. 1). The reasons of plants with ants
304 showed different patterns of herbivory between tropical and subtropical might be the
305 different links mechanism of plants and ants.

306 The Janzen-Connell hypothesis proposes that adult species gradually accrue host-
307 specific seed predators such as pathogens or herbivores, ultimately leading to higher
308 mortality of conspecifics(Janzen 1970; Connell 1971). Our results demonstrated this.
309 We observed that the incidence of conspecific seedling neighbors reduced seedling
310 survival (Fig. 2a). The effects of conspecific adults were not remarkable (Table S2),
311 though seedling survival decreased with increasing conspecific adult basal area,
312 especially in plants without ants (Fig. 2b). Furthermore, we found that plants with ants
313 had a higher survival rate than plants without ants at the same density (Fig. 2b, c), which
314 suggested that ant could mediate the strength of CNDD.

315 Plants make choices about how to allocate their resources. Defense and growth often

represent two ends of this resource trade-off spectrum(Herm & Mattson 1992). Plants which devote resources towards ant mutualists invest heavily in defense at the cost of growth. It appears, however, that this investment pays off by giving plants a relatively higher rate of survival. Ant can deter herbivores to improve plant fitness, which may in turn reduce the likelihood of pathogen incidence or massive herbivore damage(Clark *et al.* 2019). However, the associations between ants and plants could be established via herbivores in the subtropical forests. That means plants with close association with ants grow slowly because of herbivores. Our results showed that ants are not benefit to plants growth (Fig. 3a) and plants with ants exhibited lower RGR (Fig. 3b, c).

A recent meta-analysis showed that intraspecific competition is much stronger than interspecific competition in plant communities(Adler *et al.* 2018). We observed that the density of conspecific seedlings was positively associated with seedling growth, while increases in the density of heterospecific adult neighbors reduced seedling growth (Fig. 3a). This seems counter-intuitive at first, as it may suggest that a neighborhood of conspecifics improves seedling fitness. Survival, however, was negatively correlated with growth. It is our belief that the circumstances which lead to high proportion of conspecific seedlings germinating (namely light and resource access) also lead to relatively faster growth rates. This in turn, however, leads to intense intraspecific competition and ultimately lower survival rates. This result is consistent with our observations, as we found that survival and RGR were inversely proportional.

Plants associated with ants invest heavily in defense, at the cost of reduced annual growth. Unsurprisingly, we found that plants associated with ants had a generally

weaker relationship between conspecific neighbor density and survival. In other words, the investment in defensive mutualists (ants) mediated the influence of CNDD in plants. Though this investment corresponded to a reduced growth rate, plants with ant mutualists were more likely to survive – a much more meaningful measure when considering how CNDD influences community composition.

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Authors' Contribution:

X.J.Q. and M.X.J. designed the research; D.P. and Y.Z.Q. conceived ideas; G.Z. compiled and analyzed data with the help of X.J.Q.; G.Z. and X.J.Q. led the writing of the manuscript. All authors revised the drafts and gave final approval for publications.

Competing interests

The authors declare no competing interests.

References

Adler, P.B., Smull, D., Beard, K.H., Choi, R.T., Furniss, T., Kulmatiski, A., *et al.*

362 (2018). Competition and coexistence in plant communities: intraspecific
 363 competition is stronger than interspecific competition. *Ecol. Lett.*, 21, 1319–
 364 1329.

365 Bachelot, B., Alonso-Rodríguez, A.M., Aldrich-Wolfe, L., Cavaleri, M.A., Reed, S.C.
 366 & Wood, T.E. (2020). Altered climate leads to positive density-dependent
 367 feedbacks in a tropical wet forest. *Glob. Chang. Biol.*, 26, 3417–3428.

368 Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015). Fitting Linear Mixed-
 369 Effects Models Using lme4. *J. Stat. Softw.*, 67, 1–48.

370 Bixenmann, R.J., Coley, P.D. & Kursar, T.A. (2011). Is extrafloral nectar production
 371 induced by herbivores or ants in a tropical facultative ant-plant mutualism?
 372 *Oecologia*, 165, 417–425.

373 Blüthgen, N., Verhaagh, M., Goitía, W., Jaffé, K., Morawetz, W. & Barthlott, W.
 374 (2000). How plants shape the ant community in the Amazonian rainforest
 375 canopy: The key role of extrafloral nectaries and homopteran honeydew.
 376 *Oecologia*, 125, 229–240.

377 Bruna, E.M., Lapola, D.M. & Vasconcelos, H.L. (2004). Interspecific variation in the
 378 defensive responses of obligate plant-ants: Experimental tests and consequences
 379 for herbivory. *Oecologia*, 138, 558–565.

380 Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003). Inclusion of facilitation into
 381 ecological theory. *Trends Ecol. Evol.*, 18, 119–125.

382 Chen, L., Mi, X., Comita, L.S., Zhang, L., Ren, H. & Ma, K. (2010). Community-
 383 level consequences of density dependence and habitat association in a

384 subtropical broad-leaved forest. *Ecol. Lett.*, 13, 695–704.

385 Chen, L., Swenson, N.G., Ji, N., Mi, X., Ren, H., Guo, L., *et al.* (2019). Differential

386 soil fungus accumulation and density dependence of trees in a subtropical forest.

387 *Science* (80-.), 366, 124–128.

388 Clark, R.E., Farkas, T.E., Lichter-Marck, I., Johnson, E.R. & Singer, M.S. (2016).

389 Multiple interaction types determine the impact of ant predation of caterpillars in

390 a forest community. *Ecology*, 97, 3379–3388.

391 Clark, R.E., Gutierrez Illan, J., Comerford, M.S. & Singer, M.S. (2019). Keystone

392 mutualism influences forest tree growth at a landscape scale. *Ecol. Lett.*, 22,

393 1599–1607.

394 Comita, L.S. & Hubbell, S.P. (2009). Local neighborhood and species' shade

395 tolerance influence survival in a diverse seedling bank. *Ecology*, 90, 328–334.

396 Comita, L.S., Muller-Landau, H.C., Aguilar, S. & Hubbell, S.P. (2010). Asymmetric

397 Density Dependence Shapes Species Abundances in a Tropical Tree Community.

398 *Science* (80-.), 329, 330–333.

399 Comita, L.S., Queenborough, S.A., Murphy, S.J., Eck, J.L., Xu, K., Krishnadas, M., *et*

400 *al.* (2014). Testing predictions of the Janzen-Connell hypothesis: A meta-

401 analysis of experimental evidence for distance- and density-dependent seed and

402 seedling survival. *J. Ecol.*, 102, 845–856.

403 Condit, R. (1998). *Tropical Forest Census Plots*. Springer.

404 Connell, J. (1971). On the role of natural enemies in preventing competitive exclusion

405 in some marine animals and in rain forest trees. In: *den Boer PJ, Gradwell GR*

406 *eds. Dynamics of Populations*. pp. 298-312. 1971.

407 Gelman, A. & Hill, J. (2006). *Data Analysis Using Regression and*

408 *Multilevel/Hierarchical Models*. Cambridge University Press.

409 Harms, K.E., Wright, S.J., Calderón, O., Hernández, A. & Herre, E.A. (2000).

410 Pervasive density-dependent recruitment enhances seedling diversity in a tropical

411 forest. *Nature*, 404, 493–495.

412 Hazelwood, K., Beck, H. & Paine, C.E.T. (2021). Negative density dependence in the

413 mortality and growth of tropical tree seedlings is strong, and primarily caused by

414 fungal pathogens. *J. Ecol.*, 109, 1909–1918.

415 Heil, M., Fiala, B., Maschwitz, U. & Linsenmair, K.E. (2001). On benefits of indirect

416 defence: Short- and long-term studies of antiherbivore protection via mutualistic

417 ants. *Oecologia*, 126, 395–403.

418 Heil, M. & McKey, D. (2003). Protective Ant-plant Interactions as Model Systems in

419 Ecological and Evolutionary Research. *Annu. Rev. Ecol. Evol. Syst.*, 34, 425–

420 453.

421 Herm, D.A. & Mattson, W.J. (1992). The Dilemma of Plants: To Grow or Defend. *Q.*

422 *Rev. Biol.*, 67, pp.283-335.

423 Hölldobler, B. & Wilson, E.O. (1990). *The Ants*. Harvard University Press,

424 Cambridge.

425 Hunt, R. (1982). *Plant Growth Curves - The Functional Approach to Plant Growth*.

426 Edward Arnold, London, UK.

427 Ito, F. & Higashi, S. (1991). An Indirect Mutualism between Oaks and Wood Ants

428 Via Aphids. *J. Anim. Ecol.*, 60, 463.

429 Janzen, D.H. (1970). Herbivores and the Number of Tree Species in Tropical Forests.

430 *Am. Nat.*, 104, 501–528.

431 Jia, S., Wang, X., Yuan, Z., Lin, F., Ye, J., Lin, G., *et al.* (2020). Tree species traits

432 affect which natural enemies drive the Janzen-Connell effect in a temperate

433 forest. *Nat. Commun.*, 11, 1–9.

434 Jiang, F., Lutz, J.A., Guo, Q., Hao, Z., Wang, X., Gilbert, G.S., *et al.* (2021).

435 Mycorrhizal type influences plant density dependence and species richness

436 across 15 temperate forests. *Ecology*, 102, 1–11.

437 Johnson, D.J., Beaulieu, W.T., Bever, J.D. & Clay, K. (2012). Conspecific negative

438 density dependence and forest diversity. *Science* (80-.), 336, 904–907.

439 Johnson, D.J., Condit, R., Hubbell, S.P. & Comita, L.S. (2017). Abiotic niche

440 partitioning and negative density dependence drive tree seedling survival in a

441 tropical forest. *Proc. R. Soc. B Biol. Sci.*, 284.

442 Klironomos, J.N. (2002). Feedback with soil biota contributes to plant rarity and

443 invasiveness in communities. *Nature*, 417, 67–70.

444 Kobe, R.K. & Vriesendorp, C.F. (2011). Conspecific density dependence in seedlings

445 varies with species shade tolerance in a wet tropical forest. *Ecol. Lett.*, 14, 503–

446 510.

447 Lamanna, J.A., Walton, M.L., Turner, B.L. & Myers, J.A. (2016). Negative density

448 dependence is stronger in resource-rich environments and diversifies

449 communities when stronger for common but not rare species. *Ecol. Lett.*, 19,

450 657–667.

451 Lu, J., Johnson, D.J., Qiao, X., Lu, Z., Wang, Q. & Jiang, M. (2015). Density
 452 dependence and habitat preference shape seedling survival in a subtropical forest
 453 in central China. *J. Plant Ecol.*, 8, 568–577.

454 Lu, Z., Bao, D., Guo, Y., Lu, J., Wang, Q., Qiao, X., *et al.* (2013). Community
 455 Composition and structure of Badagongshan forest dynamic Plot (BDGS) in a
 456 mid-subtropical mountain evergreen and deciduous Broad-leaved mixed forest.
 457 *Plant Sci. J.*, 31, 336–344.

458 Luo, Y., Taylor, A., Weigelt, P., Guénard, B., Evan, P., Nowak, A., *et al.* (2022).
 459 Climate and ant richness explain the global distribution of ant-plant mutualisms.
 460 *bioRxiv*, 45–52.

461 Mangan, S.A., Schnitzer, S.A., Herre, E.A., MacK, K.M.L., Valencia, M.C., Sanchez,
 462 E.I., *et al.* (2010). Negative plant-soil feedback predicts tree-species relative
 463 abundance in a tropical forest. *Nature*, 466, 752–755.

464 Parker, J. & Kronauer, D.J.C. (2021). How ants shape biodiversity. *Curr. Biol.*, 31,
 465 R1208–R1214.

466 Petermann, J.S., Fergus, A.J.F., Turnbull, L.A. & Schmid, B. (2008). Janzen-Connell
 467 effects are widespread and strong enough to maintain diversity in grasslands.
 468 *Ecology*, 89, 2399–2406.

469 Pu, X., Weemstra, M., Jin, G. & Umaña, M.N. (2022). Tree mycorrhizal type
 470 mediates conspecific negative density dependence effects on seedling herbivory,
 471 growth, and survival. *Oecologia*, 199, 907–918.

472 De Queiroz, A.C.M., Da Costa, F.V., De Siqueira Neves, F. & Fagundes, M. (2013).
 473 Does leaf ontogeny lead to changes in defensive strategies against insect
 474 herbivores? *Arthropod. Plant. Interact.*, 7, 99–107.

475 R Core Team. (2020). R: A Language and Environment for Statistical Computing in
 476 R. *Found. Stat. Comput.*

477 Rico-Gray, V. & Oliveira, P.S. (2007). *The Ecology and Evolution of Ant-Plant*
 478 *Interactions*. University of Chicago Press.

479 Schroeder, J.W., Dobson, A., Mangan, S.A., Petticord, D.F. & Herre, E.A. (2020).
 480 Mutualist and pathogen traits interact to affect plant community structure in a
 481 spatially explicit model. *Nat. Commun.*, 11, 1–10.

482 Segnitz, R.M., Russo, S.E., Davies, S.J. & Peay, K.G. (2020). Ectomycorrhizal fungi
 483 drive positive phylogenetic plant–soil feedbacks in a regionally dominant
 484 tropical plant family. *Ecology*, 101, 1–15.

485 Da Silva, C.V.C., da Silva Goldas, C., Dáttilo, W., Dröse, W., de Souza Mendonça,
 486 M. & Podgaiski, L.R. (2020). Effects of time-since-fire on ant-plant interactions
 487 in southern Brazilian grasslands. *Ecol. Indic.*, 112.

488 Song, X., Yang, J., Cao, M., Lin, L., Sun, Z., Wen, H., *et al.* (2021). Traits mediate a
 489 trade-off in seedling growth response to light and conspecific density in a diverse
 490 subtropical forest. *J. Ecol.*, 109, 703–713.

491 Song, X., Zhang, W., Johnson, D.J., Yang, J., Asefa, M., Deng, X., *et al.* (2020).
 492 Conspecific negative density dependence in rainy season enhanced seedling
 493 diversity across habitats in a tropical forest. *Oecologia*, 193, 949–957.

494 Xu, M. & Yu, S. (2014). Elevational variation in density dependence in a subtropical.
495 *Ecol. Evol.*, 4, 2823–2833.

496 Zhang, J., Swenson, N.G., Liu, J., Liu, M., Qiao, X. & Jiang, M. (2020). A
497 phylogenetic and trait-based analysis of community assembly in a subtropical
498 forest in central China. *Ecol. Evol.*, 10, 8091–8104.

499 Zhu, Y., Comita, L.S., Hubbell, S.P. & Ma, K. (2015). Conspecific and phylogenetic
500 density-dependent survival differs across life stages in a tropical forest. *J. Ecol.*,
501 103, 957–966.

502 Zhu, Y., Mi, X., Ren, H. & Ma, K. (2010). Density dependence is prevalent in a
503 heterogeneous subtropical forest. *Oikos*, 119, 109–119.

504