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**Warming intensifies soil pathogen negative feedback on a temperate tree**

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Supporting information

- **Fig. S1** Average temperature of soil in open-top chambers (OTCs) and control plots (from April 2016 to August 2018).
- **Fig. S2** Distance-decay abundance of fungal pathogens from adults of *Prunus padus* and increased abundance of fungal pathogens under warming.
- **Table S1** All OTUs for 36 soil samples.
- **Table S2** Fungal OTUs for 36 soil samples.

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**Table S3** 255 OTUs classified by FUNGuild as pathogens and those 101 pathogens with confirmed virulence to *Prunus* species highlighted in yellow color.

**Table S4** Seedling survival times around the three adults of *Prunus padus*.

**Notes S1** Estimation of conspecific negative density dependence using the Ricker model.

**Notes S2** Testing the Cox proportional hazard assumption for experimental treatments.

**Notes S3** Quantifying overdispersion in the generalized linear mixed model for modeling responses of relative abundance of plant-pathogenic fungi to experimental treatments.
Summary

- The soil pathogen-induced Janzen-Connell (JC) effect is considered as a primary mechanism regulating plant biodiversity worldwide. As predicted by the framework of the classic plant disease triangle, severity of plant diseases is often influenced by temperature, yet insufficient understanding of how increasing temperatures affect the JC effect contributes uncertainty in predictions about how global warming affects biodiversity.
- We conducted a three-year field warming experiment, combining open-top chambers with pesticide treatment, to test the effect of elevated temperature on seedling mortality of a temperate tree species, *Prunus padus*, from a genus with known susceptibility to soil-borne pathogens.
- Elevated temperature significantly increased mortality of *P. padus* seedlings in the immediate vicinity of parent trees, concurrent with increased relative abundance of pathogenic fungi identified to be virulent to *Prunus* species.
- Our study offers experimental evidence suggesting that global warming significantly intensify the JC effect on a temperate tree species due to increased relative abundance of pathogenic fungi. This work advances our understanding about changes in the JC effect linked to the ongoing global warming, which has important implications for predicting tree diversity in a warmer future.

Key words: global warming, Janzen-Connell effect, plant disease triangle, *Prunus padus*, soil pathogens, temperate forest, tree species diversity, warming experiment

Introduction

Critical to predictions of how climate change affects biodiversity is understanding of the mechanisms that maintain diversity. A major mechanism found to maintain plant species diversity at the global scale from tropical to temperate forests (Augspurger, 1983; Packer & Clay, 2000; Bell et al., 2006; Mangan et al., 2010; Bagchi et al., 2014; Bever et al., 2015; Liu et al., 2015; Chen et al., 2019; Levi et al., 2019; Jia et al., 2020) as well as grasslands (Petermann et al., 2008; Semchenko et al., 2018) is the Janzen-Connell (JC) effect (Janzen, 1970; Connell, 1971), which is primarily regulated by the negative distance- or density-dependent effect inflicted by host-specific pathogens on plant species. We recognized that other enemies including herbivores could also induce the JC effect. In this study,
we exclusively focused on soil-borne pathogens. It is however an unanswered question how the JC effect may be altered by the ongoing and projected global warming (due to changes in host-specific pathogens and thus changes in severity of plant diseases). Recent studies have indicated that microbial diversity can be significantly altered under climate change, e.g., fungal diversity has increased in the Antarctic (Newsham et al., 2016) and bacterial population size and richness has changed in grasslands (Sheik et al., 2011). Further, warming can increase the relative abundance of soil fungal pathogens globally (Delgado-Baquerizo et al., 2020), thereby influencing plant-microbiota interactions (Hutchins et al., 2019; Liu & He, 2019).

The occurrence of plant diseases in any system must involve a susceptible host, a virulent pathogen and amenable environmental conditions, as formulated by the classic disease triangle model of plant pathology (Francl, 2001). As such, climate change (e.g., global warming) as a risk factor is expected to inevitably change host-pathogen interactions (Chakraborty, 2013; Launay et al., 2014; Liu & He, 2019), and under certain conditions non-virulent fungi can even become virulent and cause plant diseases (Chakraborty et al., 2000; Barford, 2013). Although the effects of warming on plant diseases have been widely studied in an agricultural context (Tapsoba & Wilson, 1997; Siebold & von Tiedemann, 2013; Launay et al., 2014), it is only recently that attention has started to be paid to warming effects on natural ecosystems in the context of biodiversity maintenance (Swinfield et al., 2012; van der Putten et al., 2016; Liu et al., 2019; Milici et al., 2020). For example, a six-year warming experiment showed that increased temperature was a primary driver for the observed increase in foliar pathogen loads in a meadow ecosystem in the Tibet plateau (Liu et al., 2019).

Another in situ warming experiment in a wet tropical forest in Puerto Rico reported that the effect of conspecific negative density-dependent (CNDD) on seedling survival changed to be positive after three years of warming (presumably due to decreased pathogen pressure or other processes) (Bachelot et al., 2020). This empirical result made the authors suggest that diversity of wet tropical forests could decline under global warming because of changes in CNDD (Bachelot et al., 2020). It however remains unclear how this result may be extrapolated to other forest ecosystems, particularly forests at higher latitudes where the intensity and trend of global warming are very different from that in the tropics (IPCC, 2013).
Here, we conducted a field warming experiment to test the effect of elevated temperature on the infestation of soil pathogens on seedlings of *Prunus padus* (L.) Gilib (Rosaceae) in a temperate forest in China (Fig. 1a). *P. padus*, commonly named as bird cherry or hackberry, is native to northern Asia and northern Europe and grows up to about 16 m tall. The genus of *Prunus* is widely known as susceptible to soil-borne pathogens (inducing seedling damping-off) (Agrios, 2005) and is a “model” genus used for testing pathogen-regulated JC effects in temperate forests (Packer & Clay, 2000; Reinhart & Clay, 2009; Bennett et al., 2017). We used open-top chambers (OTCs) to simulate warming (see Materials and Methods; Fig. 1b). The experiment involved three factors: warming (2 levels of treatment: OTC warming versus control), distance to parent trees (3 distances: 1, 10 and 20 m away from a *P. padus* parent tree) and pesticide treatment (watering with pesticides versus without).

For the purpose of comparison and for testing the extent of host-specificity for the pathogens on *P. padus* seedlings, we also included seedlings of two other common tree species in this forest, *Malus baccata* (L.) Borkh (Rosaceae) and *Quercus mongolica* Fischer ex Ledebour (Fagaceae) (Fig. 1b), in the experiment. The experiment lasted for three years (June 2016 to September 2018). Seedling survival was censused every two weeks in the first two years and every six weeks in the third year. At the end of the experiment, we tested whether warming increased the relative abundance of fungal pathogens, which are assumed to be responsible for the mortality of their host seedlings (Chen et al., 2019). Moreover, we further used the Ricker model to assess whether *P. padus* population in the study forest experienced CNDD (LaManna et al., 2017, 2021).

**Materials and Methods**

**Study site**

Our study site is located in the Changbaishan Nature Reserve (43°23′ N, 128°5′ E) in northeastern China (Fig. 1a). It is a temperate coniferous and broad-leaved mixed forest. Experiments were established adjacent to a 25 ha stem-mapping forest plot, where all stems with diameter at breast height (DBH) ≥ 1 cm were mapped in 2004, and the plot has since been recensused every five years (Notes S1) (Wang et al., 2010).
**Experimental design**

Our experiment considered three factors: warming, pesticide treatment and distance to parent trees, as described below. The study tree species is *P. padus*. The species was selected because it is native, locally abundant and from a genus with known susceptibility to soil pathogens (Packer & Clay, 2000; Reinhart & Clay, 2009; Bennett *et al.*, 2017). In addition to this focal species, two other locally abundant tree species, *M. baccata* and *Q. mongolica*, were also used in the experiment to confirm host-specificity of the pathogens. If the seedlings of those two species would not show similar responses to pesticide treatment and/or distance to *P. padus* adults as *P. padus* seedlings, it would suggest specificity of pathogens to *P. padus*, which is a critical condition for the JC hypothesis (Janzen, 1970; Connell, 1971; Freckleton & Lewis, 2006).

Three adult *P. padus* trees with DBH > 15 cm were randomly selected with the condition that no other *P. padus* adult trees were between them within an area of about 1 ha. In September 2015, three open-top chambers (OTCs with size of 160×160×160 cm; made of 4 mm thick transparent Plexiglas boards with light transmission > 92% between 250 and 800 nm wavelength; Plexiglas, Evonik Degussa, Essen, Germany) (Liu *et al.*, 2011) were deployed at distances of 1, 10 and 20 m away from each selected adult tree in such a way that the focal adult tree was the nearest conspecific adult to any OTC (9 OTCs in total; Fig. 1b). Beside each OTC, a 160×160 cm control plot was also established. The control plots were fenced by 50 cm tall 5-mm steel screen to prevent animals from trampling or foraging. In this study, OTCs caused a (passive) warming of 1.2 ± 0.5 °C (OTCs were used because active warming with eclectic heaters is forbidden in the nature reserve), which was consistent with the magnitude of soil warming commonly observed for OTCs in temperate forests of northern Europe and Qinghai-Tibet Plateau forests of China (0.4–1 °C) (De Frenne *et al.*, 2011; Li *et al.*, 2015), though being relatively low compared to that in open-field ecosystems (e.g., tundra and alpine meadow) (Hollister & Webber, 2000; Liu *et al.*, 2011). Given that the global average rise in temperature is already 0.85 °C above the preindustrial level, the additional warming achieved in this study approaches or slightly exceeds the critical upper limit of 2 °C warming above the preindustrial levels set at the COP21 Talks in Paris in 2015. Thus, the OTCs in this study provided a realistic warming emulation of projected global warming.
Within each OTC six 40×40 cm quadrats were established with a two columns layout (Fig. 1b). Seedlings of the focus species (*P. padus*) and two other species (*M. baccata* and *Q. mongolica*), were each transplanted to two quadrats as shown in Fig. 1b. One column of three quadrats was randomly selected to receive pesticide treatment and the other three quadrats were treated with the same amount of sterilized clean water without pesticides. The pesticide treatment included two selective pesticides (‘Celest Gold’ and ‘Ridomil Gold’; Syngenta Ltd, Basel, Switzerland). ‘Celest Gold’ is effective in controlling pathogenic fungi (e.g., *Fusarium* spp., *Rhizoctonia* spp., *Septoria* spp., and so on) causing seedling damping-off and leaf spot, a major agent of seedling death (Liu *et al.*, 2015), and ‘Ridomil Gold’ is for controlling oomycete pathogens (e.g., *Pythium* spp.) that also cause seedling damping-off (Bell *et al.*, 2006). These two specific pesticides were administered by spray, every two weeks for the first two years and every six weeks thereafter. According to the manufacturer’s recommendations, the amounts used for the two pesticides were 0.25 g m\(^{-2}\) of granular Ridomil Gold dissolved in 1 L of sterilized clean water and 0.50 g m\(^{-2}\) liquid Celest Gold diluted in 1 L of sterilized clean water.

Quadrats without pesticide treatment were sprayed with the same amount of sterilized clean water. Seedling mortality was recorded whenever pesticides or watering was applied.

In early June 2016, two-week old seedlings, germinated in sterilized sands in greenhouse from seeds collected from same forest in the autumn of 2015 (seeds of *P. padus* were collected from the parent trees), were transplanted to the 40×40 cm quadrats at a density of 9 seedlings per quadrat (Fig. 1b). The same design and treatments were repeated for the control (non-OTC) plots.

In total, there were 36 combinations of experimental treatments (3 adult trees × 2 levels of warming treatment × 2 levels of pesticide treatment × 3 distances).

**Soil temperature and moisture**

Soil temperature and moisture were monitored in the OTC and control plots using microclimate data loggers, with TM sensors inserted 10 cm deep in the soil (Em50-5TM; Decagon Devices Inc., Pullman, WA, USA). Soil temperature and moisture data were recorded every four hours, starting in middle April each year when seeds began to germinate in the field and ceased in November (when temperature fell below 0 °C and soil became inactive; Fig. S1) in the first two years. In the third year (2018), soil temperature and moisture measurement were stopped at the end of August (the field
experiment ended on September 1, 2018; Fig. S1). OTCs had no significant effect on soil moisture ($t = 0.65$, $df = 18$, and $p = 0.52$ using a paired $t$-test).

**Molecular analyses of soil fungal community**

Soil cores were collected near the end of our three-year experiment (June 2018) for molecular analyses of soil fungi. Briefly, bulk soils were sampled at the interstices between transplanted seedlings of all three species (Fig. 1b). Assessment of fungal communities directly associated with roots of seedlings (which might be more directly associated with seedling mortality) was not feasible because no roots could be found for seedlings that died in the first two years (or even for seedlings that died in April of the third year). After removal of surface debris, soil from 0-10 cm in depth were collected using a soil auger with a 5-cm inner diameter, which was washed thoroughly with double distilled water after each sampling. In total, one soil sample was collected for each of the 36 combinations of treatments. Separate soil samples were not collected for each of the three seedling species because the seedlings were transplanted from the germination in sterilized sand in greenhouse.

The sampled soils were immediately frozen and shipped on dry ice to the laboratory of Magigene Technology Ltd. in Guangzhou and were stored at -80°C prior to DNA extraction. Fungal DNA was extracted from 1 g of soil using the PowerSoil® DNA isolate kit (MO BIO Laboratories Incorporation, Carlsbad, CA, USA). The concentration and purity of the extracted DNA samples were measured using the NanoDrop One (Thermo Fisher Scientific, MA, USA). The ITS region 2 was amplified with primers ITS3 (5’- GCATCGATGAAGAACGCAGC-3’) and ITS4 (5’- TCCTCCGCTTATTGATATGC-3’) and sequenced on an Illumina MiSeq/Hiseq 2500 platform, generating 250 bp paired-end reads.

Sequencing data processing included paired-end raw reads quality control according to the Trimmomatic (Bolger et al., 2014), paired-end clean reads assembly using FLASH (Magoč & Salzberg, 2011), and raw tags quality control using the Mothur software (Schloss et al., 2009). Using USEARCH (Edgar, 2010), sequences with 97% similarity were clustered into operational taxonomic units (OTUs), and chimera and singleton OTUs were removed. The clustering cutoff threshold at a sequence similarity of 97% is recognized as an appropriate compromise that takes intraspecific and interspecific sequence variation and random sequencing errors into consideration (Tedersoo et al., 2019). The UNITE fungal database (https://unite.ut.ee; Kõljalg et al., 2013) was used for taxonomic
annotation of fungal OTUs, using the most abundant sequence type for BLAST searches (Nilsson et al., 2019). There were 8,438 OTUs in total, of which 74% (6253) were fungi (39% of these were identified to the genus level), and the rest were Chromista (8%), Plantae (6%), Protista (1%), Protozoa (0.06%), unidentified (1%) or without BLAST hit (9%) (Table S1). A table of fungal OTUs detected in each sample is provided in Table S2.

Fungal OTUs identified to the genus level were further assigned to guilds (i.e., plant-pathogenic fungi, arbuscular mycorrhizal fungi, and so on) using the FUNGuild database (Nguyen et al., 2016) (http://www.stbates.org/guilds/app.php; accessed in December 2019). We also referred to the latest literature (Mommer et al., 2018) for further assessment of trophic strategies. We only identified OTUs as plant pathogens if they could be designated as pathogens with a confidence level of “probable” or “highly probable” (Nguyen et al., 2016) in FUNGuild, excluding mixed modes (Delgado-Baquerizo et al., 2020). A list of 255 plant-pathogenic fungi identified from the FUNGuild database is provided in Table S3.

The classification of functional guilds by the FUNGuild database is coarse, and the 255 pathogenic fungi designated by the database may not all be pathogenic to P. padus. To confirm their pathogenicity to the host, we further searched published sources for fungi that were reported to be pathogenic on Prunus species. This search primarily included the database of the American Phytopathological Society (https://www.apsnet.org/Pages/default.aspx) that maintains a large number of economically important plant diseases in the genus Prunus (e.g., cultivated fruits or ornamentals) that have been well studied for pathogens. We also referred to Web of Science literature searches (TOPIC “the genus name of pathogenic fungi” AND “plant disease”) (see Table S3). We were able to confirm 101 out of the 255 FUNGuild pathogenic OTUs as specific to Prunus species. The remaining 154 pathogenic OTUs could still (most likely) contain fungi virulent to P. padus. However, we expected that the 101 Prunus specific fungi would impose a stronger distance effect on P. padus seedlings. To test that, we included analyses to quantify and compare this as well as the effect of all pathogenic fungi on seedling survival.

Although the pesticide treatment also targeted oomycete pathogens (as shown in prior work in the genus Prunus; Packer & Clay, 2000), our community analysis exclusively focused on fungal pathogens not including oomycetes. The reason was that the ITS primers used in this study were not
able to classify oomycetes; all of them were grouped as Chromista (chromistan fungal analogues; Table S1). Even though improved oomycete-specific primers are currently available (Legeay et al., 2019), these primers can only be used to qualitatively determine oomycete diversity, not their abundance.

**Statistical analyses**

*Analysis of seedling survivorship*

Most studies that test the pathogen-induced JC effect do not record survival time of individuals but simply count live and dead plants at the end of an experiment (but see Spear et al., 2015). We recorded survival times of individual seedlings over the three-year experiment period (June 2016–September 2018; Table S4), yielding more powerful data for statistical inferences. We first estimated and tested the difference of survivorship for *P. padus* seedlings under different treatments (Fig. 2). Because the survival times involved censored observations, i.e., those seedlings that were still alive at the time of observation, the product limit estimate (Keplan-Meier estimate) of survival function should be used (Lawless, 2002). The survivorship curves were estimated using the R function ‘survfit’ of the ‘survival’ package (http://survival.r-forge.r-project.org/). Survivorship curves were also estimated for the non-target species *M. baccata*, and *Q. mongolica*. Because no difference in survival probability was found for these two species across different treatments (Fig. 2), no further analyses were conducted.

We used Cox proportional hazards (PH) models to model hazard ratios of *P. padus* seedling mortality under experimental treatments (i.e., warming and pesticides) for survival times by the end of the first, second and third year of the experiment, respectively. Because no effect of fungal pathogens on survival of *P. padus* seedlings was detected at 10 and 20 m distances, the Cox PH model was only applied to seedlings at 1 m distance. The three parent trees were treated as a “cluster” in the ‘coxph’ function of the R package ‘survival’ (http://survival.r-forge.r-project.org/). The PH assumption was tested for all the experimental treatments in the three time periods. The assumption was met for the first and second year but not for the third year, for which the accelerated failure time model was used (Notes S2).
Analysis of the relative abundance of plant-pathogenic fungi

To test whether the relative abundance of plant-pathogenic fungi (all 255 OTUs classified as pathogens by FUNGuild and the 101 Prunus specific pathogens, respectively; Table S3) responded to experimental treatments, we used a generalized linear mixed effect model (GLMM) to quantify the effects of warming, pesticide treatment, distance from P. padus adults and their interaction terms on the relative abundance of each group of fungal pathogens. The relative abundance was calculated by dividing sequence counts of plant pathogens by the total sequence counts of fungal OTUs in a sample. The three adult individuals of P. padus were treated as a random effect. Overdispersion in the data was tested using ‘overdisp_fun’ (see Notes S3). Because there was a sign of overdispersion ($p < 0.001$), a negative binomial GLMM was used with the ‘glmmTMB’ function of the R package glmmTMB (see Notes S3), with the total sequence counts of fungal OTUs in each sample as “weights”.

Hazard risk of seedling mortality associated with the increase in the relative abundance of pathogenic fungi

To test the effect of the relative abundance of pathogenic fungi (all 255 FUNGuild classified OTUs and the 101 Prunus OTUs, respectively; Table S3) on hazard risk of P. padus seedling mortality, the Cox PH model was also used to model hazard of P. padus seedling mortality associated with the relative abundance of plant pathogens up to the end of the experiment. Because of no effect of fungal pathogens on the survival of P. padus seedlings at 10 and 20 m distances (Fig. 2b, c), which is consistent with previous findings that the JC effect typically acts at the neighborhood no more than 10 meters away from adults (Liu et al., 2012; Murphy et al., 2017), we modeled data at 1 m distance only. Again, the three parent trees were treated as a “cluster” in the ‘coxph’ function. We repeated the same analysis on the seedlings of M. baccata, and Q. mongolica. The PH assumption for the relative abundance of pathogenic fungi was met for all three species ($p$ at least > 0.57).

Estimating CNDD in P. padus population

We used the Ricker model to quantify the CNDD strength for P. padus in the 25 ha Changbaishan stem-mapping plot (Wang et al., 2010) following LaManna et al. (2017). Stems of P. padus were classified as either adults or saplings. Because P. padus is of smaller stature, we used diameter at breast height (DBH) = 6.2 cm as the threshold (the third quartile of its DBH size). For other (non-
focal) species, DBH = 10 cm was used as the threshold to categorize adults and saplings following LaManna et al. (2017). The 25 ha plot was divided into 20×20 m quadrats and adult trees and saplings were counted in each quadrat (see Notes S1 for the set-up of the Ricker model). As the approach of LaManna et al. (2017) has been criticized in the literature (Detto et al., 2019), we reanalyzed the CNDD of *P. padus* with a distance-weighted approach by incorporating adult abundance and a dispersal-kernel null model (LaManna et al., 2021). Because both analyses detected a strong CNDD signal, we only reported the CNDD result of the Ricker model.

**Results**

**Seedling survivorship**

The Kaplan-Meier survivorship curves showed that seedlings of *P. padus* at 1 m distance suffered a significantly higher mortality under warming than in the control treatment (*p* < 2e-16, Fig. 2a), while this warming effect disappeared after pesticides were sprayed to suppress fungal and oomycete pathogens (*p* = 0.79, Fig. 2a). As further evidence corroborating the JC effect, the pesticide treatment dramatically increased the survivorship of *P. padus* seedlings beneath the parent trees both for the warming treatment (*p* = 2e-11, Fig. 2a) and for the control (*p* = 8e-5, Fig. 2a). No significant effects of warming or pesticide treatment on survivorship of *P. padus* seedlings were detected at 10 or 20 m distance (at least *p* > 0.12, Fig. 2b and c). In contrast, *M. baccata* and *Q. mongolica* seedlings did not show any differentiated response to warming or pesticide treatment at any distance around *P. padus* parent trees (at least *p* > 0.16, Fig. 2d-i).

**Hazard ratios of seedling mortality under treatments**

Tested by Cox’s proportional hazard models or an accelerated time model for seedling mortality in the plots at 1 m distance around *P. padus* parent trees, the hazard ratios (HRs) of *P. padus* seedling mortality under warming were significantly larger than 1 (HR = 1.85 after one year, *p* = 1e-9, Fig. 3a; HR = 1.70 after two years, *p* < 2e-16; Fig. 3b; HR = 1.70 after three years, *p* = 3e-15, Fig. 3c). In consistency with the survivorship curves of Fig. 2, pesticide treatment strongly reduced the hazards of seedling mortality across years (at least *p* < 5e-5, Fig. 3).

**Relative abundance of pathogenic fungi under treatments**

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OTC warming had a significantly positive effect on the relative abundance of pathogens, while pesticide treatment decreased their relative abundance (Table 1). There was a strong distance-decay in the relative abundance of these pathogens from *P. padus* parent trees (Table 1; Fig. S2), supporting the JC hypothesis that pathogens tend to accumulate in the vicinity of host parent trees. It is important to note that the distance effect on the 101 confirmed *Prunus* pathogens was stronger than that on all 255 FUNGuild classified pathogens (*z*-value = -29.9 versus -11.0), although distance effects on both groups of pathogens were highly significant (Table 1).

**Hazard risk of seedlings associated with relative abundance of pathogenic fungi**

The high relative abundance of pathogens at 1 m distance is expected to increase the mortality risk of *P. padus* seedlings but not to affect the seedlings of other two species if those pathogens are specific to *P. padus*. The results of the Cox PH models in Fig. 4 confirm this expectation, with a significantly increased hazard ratio (*p < 2e-16*) for *P. padus* seedlings associated with the increase in the relative abundance of fungal pathogens. The results in Fig. 4 further show that the effect of the 101 pathogens specific to *P. padus* was stronger than that of the 255 OTUs classified by FUNGuild as pathogens, with a 22.5% increase in *P. padus* seedling mortality for one percent increase in the relative abundance of the *Prunus* specific pathogens (Fig. 4b) but only a 12.8% increase in seedling mortality for one percent increase in the relative abundance of the 255 FUNGuild classified pathogens (Fig. 4a). In contrast, the mortality hazard ratios for the seedlings of the other two non-focal species were not significantly larger than 1 over the three years of experiment (*p > 0.08*; Fig. 4).

**Estimation of CNDD for the *P. padus* population**

The result of the Ricker model indicated that *P. padus* experienced a significant CNDD (observed value = -0.20 ± 0.03; *t* = -6.10, *p = 2e-9).

**Discussion**

The JC effect has been considered to be a primary CNDD mechanism that maintains tree diversity in tropical forests (Augspurger, 1984; Bell *et al.*, 2006; Mangan *et al.*, 2010; Bagchi *et al.*, 2014) since it was proposed in the early 1970s (Janzen, 1970; Connell, 1971). In temperate forests, CNDD has also been shown to play an important role (Hille Ris Lambers *et al.*, 2002) and many temperate tree species are subjected to negative soil pathogen feedback (Bennett *et al.*, 2017). Our study is the first
of its kind to test for changes in the JC effect under warming in temperate forests and contributes to understanding of effects of climate change on tree diversity. We showed that soil-borne fungal and oomycete pathogens benefited from elevated temperatures, supposedly resulting in increased seedling mortality of the temperate tree species *P. padus* in the immediate vicinity of their conspecific adults, thus intensifying the JC effect. In the analysis of pathogen community, warming increased the relative abundance of fungal pathogens (Table 1; Fig. S2) that were shown to increase hazard ratio for *P. padus* seedlings (Fig. 4). For oomycete pathogens, they were suppressed by pesticide treatment but could not be analyzed by sequencing because of the infeasibility of the ITS primers in classifying oomycetes. The change in plant-pathogen interaction under warming strengthens negative soil feedback and should have important implications for predictions of (indirect) effects of climate change on biodiversity (van der Putten *et al.*, 2016). Else being equal, together with the widely observed northward migration of species (Parmesan & Yohe, 2004), the intensified JC effect as documented in our study predicts that tree diversity in temperate forests would increase with global warming.

The genus *Prunus* is widely known as susceptible to soil-borne pathogens and is a “model” genus for testing pathogen-regulated JC effects (Packer & Clay, 2000; Reinhart & Clay, 2009; Bennett *et al.*, 2017). Similar to other congeners, e.g., *P. serotina* and *P. virginiana* (Packer & Clay, 2000; Reinhart & Clay, 2009; Bennett *et al.*, 2017), recruitment of *P. padus* seedlings is checked by soil pathogens. If the JC effect indeed regulates *P. padus* population as our experiment showed, we should be able to detect conspecific negative density dependence (CNDD) of this tree species in the 25 ha stem-mapping plot where our experiment took place. Using the Ricker model (LaManna *et al.*, 2017; see Notes S1 for estimation of CNDD), we indeed detected a strong CNDD for *P. padus* in the plot, consistent with the observation that seedling recruitment of *P. padus* was checked by soil-borne pathogens. Although this result does not imply that the JC effect exclusively drives the CNDD (e.g., competition could also cause CNDD), the heightened JC effect under warming would likely intensify CNDD, promoting tree species diversity in temperate forests (Johnson *et al.*, 2012; Bever *et al.*, 2015; LaManna *et al.*, 2017).

Increased severity of plant diseases under warming (Olofsson *et al.*, 2011) could arise from proliferation of soil-borne pathogens (Scherm & van Bruggen, 1994; Chakraborty, 2013; Bebber *et al.*, ...
increased virulence (Liu & He, 2019), decreased resistance of host plants (De Frenne et al., 2011), or a combination of these effects (Liu & He, 2019). Climate warming has been widely shown to exacerbate crop diseases and expand the range of plant pathogens in agricultural systems (Harvell et al., 2002; Bebber et al., 2013; Chakraborty, 2013). Climate change is also shown to alter plant-microbe interactions in natural ecosystems (Rudgers et al., 2014; Hutchins et al., 2019; Liu et al., 2019; Liu & He, 2019; Milici et al., 2020). In our study site, daily average temperatures in late April, when seeds start to germinate, are typically < 5°C and only attain a maximum around 19°C in August (Fig. S1), well below the optimum temperature (20 to 25°C) for fungal pathogen reproduction and infection (Scherm & van Bruggen, 1994). Therefore, a small increase in temperature could result in unexpectedly high seedling mortality due to increased abundance of soil-borne plant fungal pathogens (Table 1). The optimal temperature range for pathogen reproduction and infection likely explains the contrasting result of our study from that of Bachelot et al. (2020), which found that warming weakened plant-soil feedback. In their study, an increase of 4°C made temperature to exceed the upper optimal range and was deleterious to the soil pathogens in the tropics.

The classification of functional roles of the fungal species in this study was based on the database FUNGuild (Nguyen et al., 2016). Functional classification of fungi is a challenge and FUNGuild is considered to be a coarse classification, although the database has been increasingly adopted for classifying fungal guilds (Leff et al., 2018; Mommer et al., 2018; Semchenko et al., 2018; Chen et al., 2019; Delgado-Baquerizo et al., 2020). Among the pathogenic fungi classified by the FUNGuild database, we went further to identify, from independent published sources, 101 OTUs that had been confirmed virulence to Prunus species. This latter group showed a stronger distance-decay in their relative abundance with respect to P. padus adult trees than the full FUNGuild pathogens (Table 1; Fig. S2). The effect of the specified Prunus pathogens on the mortality hazard of P. padus seedlings was also stronger (Fig. 4). These results lend strong evidence in support of the finding that climate warming strengthens the JC effect in the temperate forest, causing increased mortality of P. padus seedlings in the proximity of conspecific adults (Fig. 2a; Fig. 4).

Despite the evidence for an effect of warming on the JC effect, some cautions are in place. Our sequencing method could suffer the similar problems as those summarized by Taylor et al. (2016), e.g., variable rDNA copy numbers among fungi, PCR biases, Illumina sequencing biases and
variation in the efficiency of DNA extraction among fungi. Care should be taken to estimate abundance of fungi via this method. In this study, following the suggestion of Taylor et al. (2016), we sequenced the highly variable ITS region 2 with a full consideration of these limitations. Our results were consistent with a recent study that observed increased relative abundance of fungal pathogens with warming based on Illumina amplicon ITS (region 2) sequencing of bulk soil fungi (Delgado-Baquerizo et al., 2020). It is also worthwhile noting that although 1 m from parent trees of *P. padus* (with canopy radius 2-3 m) is the distance often used for testing the JC effect beneath tree species (e.g., Augspurger, 1984; Mangan et al., 2010; Liu et al., 2015), stronger evidence would be garnered if other intermediate distances, e.g., 5 m away from parent trees could also be used. Such distances would ensure that seedlings have reduced competition from parent trees compared to those at 1 m distance but are still subjected to the effect of host-specific pathogens compared to those at 10 m distance or farther. In our study, the observation that *P. padus* seedlings at 1 m showed a strong JC effect while the seedlings of other two non-focal species did not is strong evidence indicating that *P. padus* specific pathogens are at work.

Our study highlights the importance of mechanistic understanding of climate warming effect on species diversity, to complement the many studies that focus on quantifying the magnitude of biodiversity change. The increased intensity of the soil pathogen-induced JC effect under elevated temperatures as documented in this study is expected to increase tree species diversity in temperate forests. As a caveat, it is worth noting that temperature is not the sole factor regulating soil pathogens (Sturrock et al., 2011; Swinfield et al., 2012; Veresoglou et al., 2013; Liu & He, 2019; Milici et al., 2020). Climate change and other global change factors (e.g., nitrogen deposition) and disturbance regimes (e.g., wildfires; Day et al., 2019) could inevitably complicate the effect of many other biotic and abiotic factors (e.g., soil pH, soil nutrients and precipitation) on soil microbial communities, fungal pathogens included, and thereby alter the effect of the JC process. It is a pressing need to expand the current study to other species, climatic zones and global change factors in the light of the latest UN emission gap report (https://www.unenvironment.org/resources/emissions-gap-report-2019).

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**Author Contributions**

FH and YL conceived the study. YL conducted the experiment. YL and FH analyzed the data, interpreted the results, and wrote the manuscript.

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Table 1: Generalized negative binomial linear mixed effect model for modeling the effects of warming, pesticide treatment and distance away from Prunus padus adult trees on the relative abundance of FUNGuild classified pathogens (255 fungal OTUs) and 101 specified Prunus pathogens.

| Effects          | 255 FUNGuild classified pathogens | 101 specified Prunus pathogens |
|------------------|----------------------------------|-------------------------------|
|                  | Coef (SE) z-value P(>|z|)        | Coef (SE) z-value P(>|z|)     |
| Intercept        | 6.85 (0.12) 55.3 <2e-16          | 6.55 (0.14) 45.4 <2e-16      |
| Warming          | 0.60 (3e-3) 173.5 <2e-16         | 0.51 (4e-3) 139.8 <2e-16     |
| Pesticide        | -1.19 (3e-3) -342.6 <2e-16       | -1.15 (4e-3) -318.7 <2e-16   |
| Distance         | -2e-3 (2e-4) -11.0 <2e-16        | -0.01 (2e-4) -29.9 <2e-16    |
| Warming×Pesticide| -1.21 (4e-3) -271.4 <2e-16       | -1.46 (5e-3) -311.4 <2e-16   |
| Warming×Distance | -0.07 (3e-4) -276.3 <2e-16       | -0.06 (3e-4) -226.3 <2e-16   |
| Pesticide×Distance| 0.08 (3e-4) 286.6 <2e-16       | 0.07 (3e-4) 270.2 <2e-16     |
| Warming×Pesticide×Distance| 0.02 (3e-4) 49.2 <2e-16 | 0.01 (4e-4) 39.7 <2e-16      |

The model coefficients measure the effect size of the treatments. The random effect controls for the variation associated with the three adult trees and its variance = 0.05 and 0.06, respectively.
Figure Legends

Fig. 1 (a) Location of the study site in Changbaishan Nature Reserve (a temperate forest; 128°5' E, 42°23' N) in northeastern China. (b) Establishment of the experiment in the field, showing open-top chambers (OTCs). For pesticide treatment, two pesticides (‘Celest Gold’ and ‘Ridomil Gold’) were administered by spraying to control soil pathogens causing seedling damping-off. Quadrats without pesticides were sprayed with same amount of water. (c) The warming magnitude of OTCs, showing the difference between soil temperature within OTCs and soil temperature in the corresponding control plots (monthly averages with 95% bootstrapped confidence intervals). The horizontal dashed line indicates the mean value of the warming magnitude across all months (1.2 °C).

Fig. 2 Survivorship of seedlings. Kaplan-Meier survival curves for Prunus padus seedlings (top row), Malus baccata seedlings (middle row) and Quercus mongolica seedlings (bottom row). The curves represent four treatment combinations of pesticide and warming at 1 m distance (a, d, g), 10 m distance (b, e, h), and 20 m distance (c, f, i) from the three adult trees of P. padus for the three-year field experiment. Only at the 1 m distance was warming detected to significantly reduce survival probability of P. padus seedlings, while the warming effect disappeared after pesticide treatment (see p-values in the main text). No warming or pesticide treatment effect on seedling survival was detected at 10 and 20 m distances. The vertical dashed grey lines indicate the 14th, 66th and 116th week (i.e., the end of the growing season of the 1st, 2nd and 3rd year). CK is for control.

Fig. 3 Hazard ratios (relative risk) of Prunus padus seedling mortality. The effects of warming and pesticide treatment on seedling mortality risk based on hazard models for modeling seedling survival time up to the end of the first year (a), the second year (b), and the third year (c) of the experiment. (a) and (b) were modeled using Cox proportional hazard models, while (c) was modeled by an accelerated time model (Weibull regression). In one box with the same color, a treatment and its control (CK) were included. A hazard ratio larger than one indicates an increased hazard of seedling mortality under a treatment versus control (i.e., the baseline reference). n is the number of seedlings per treatment.
Fig. 4 Hazard ratios (relative risk) estimated from Cox proportional hazard (PH) models for *Prunus padus*, *Malus baccata* and *Quercus mongolica* seedling mortality associated with the relative abundance of plant pathogenic fungi. (a) for 255 FUNGuild classified pathogenic open-top chambers (OTCs), and (b) for 101 pathogens with confirmed pathogenicity to the genus *Prunus*. Hazard ratios were estimated based on seedling survival times by the end of the three-year field experiment.
1. Prunus padus
2. Malus baccata
3. Quercus mongolica
(a) One year

| Treatment          | Hazard Ratio | p-value   |
|--------------------|--------------|-----------|
| CK (n = 54)        |              |           |
| OTC (n = 54)       | 1.85 (1.52-2.25) | 1e-9     |
| Non-pesticide (n = 54) | 0.07 (0.02-0.22) | 5e-5     |
| Pesticide (n = 54) | 1.70 (1.45-1.99) | 2e-15    |

(b) Two years

| Treatment          | Hazard Ratio | p-value   |
|--------------------|--------------|-----------|
| CK (n = 54)        |              |           |
| OTC (n = 54)       | 1.70 (1.50-1.93) | 2e-16    |
| Non-pesticide (n = 54) | 0.09 (0.05-0.17) | 3e-14    |
| Pesticide (n = 54) | 1.70 (1.45-1.99) | 2e-15    |

(c) Three years

| Treatment          | Hazard Ratio | p-value   |
|--------------------|--------------|-----------|
| CK (n = 54)        |              |           |
| OTC (n = 54)       | 1.70 (1.45-1.99) | 2e-15    |
| Non-pesticide (n = 54) | 0.25 (0.17-0.38) | 9e-10    |
| Pesticide (n = 54) | 1.70 (1.45-1.99) | 2e-15    |
(a) 

- *Prunus padus*
  - Hazard ratio: 1.13
  - 1.10-1.16; $p < 2e-16$

(b) 

- *Malus baccata*
  - Hazard ratio: 1.03
  - 1.01-1.08; $p = 0.08$

- *Quercus mongolica*
  - Hazard ratio: 1.05
  - 0.99-1.10; $p = 0.09$