New Phytologist

Research

Warming intensifies soil pathogen negative feedback on a temperate tree

Yu Liu[1,2] and **Fangliang He**[1,3]

[1]ECNU-Alberta Joint Laboratory for Biodiversity Study, Tiantong National Station for Forest Ecosystem Research, School of Ecology and Environmental Sciences, East China Normal University, Shanghai 200241, China; [2]Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, China; [3]Department of Renewable Resources, University of Alberta, Edmonton, Alberta, T6G 2H1, Canada

Author for correspondence:
Fangliang He
Email: fhe@ualberta.ca

Received: *14 February 2021*
Accepted: *7 April 2021*

New Phytologist (2021) **231:** 2297–2307
doi: 10.1111/nph.17409

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

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 3-yr field warming experiment, combining open-top chambers with pesticide treatment, to test the effect of elevated temperatures on seedling mortality of a temperate tree species, *Prunus padus*, from a genus with known susceptibility to soil-borne pathogens.
- Elevated temperature significantly increased the 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 intensifies 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 ongoing global warming, which has important implications for predicting tree diversity in a warmer future.

Introduction

Critical to predictions of how climate change affects biodiversity is an 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-dependent or density-dependent effect inflicted by host-specific pathogens on plant species. We recognised 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 therefore changes in severity of plant diseases). Recent studies have indicated that microbial diversity can be significantly altered under climate change, for example fungal diversity has increased in the Antarctic (Newsham *et al.*, 2016) and bacterial population size and richness have changed in grasslands (Sheik *et al.*, 2011). Furthermore, 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 nonvirulent 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 6-yr 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

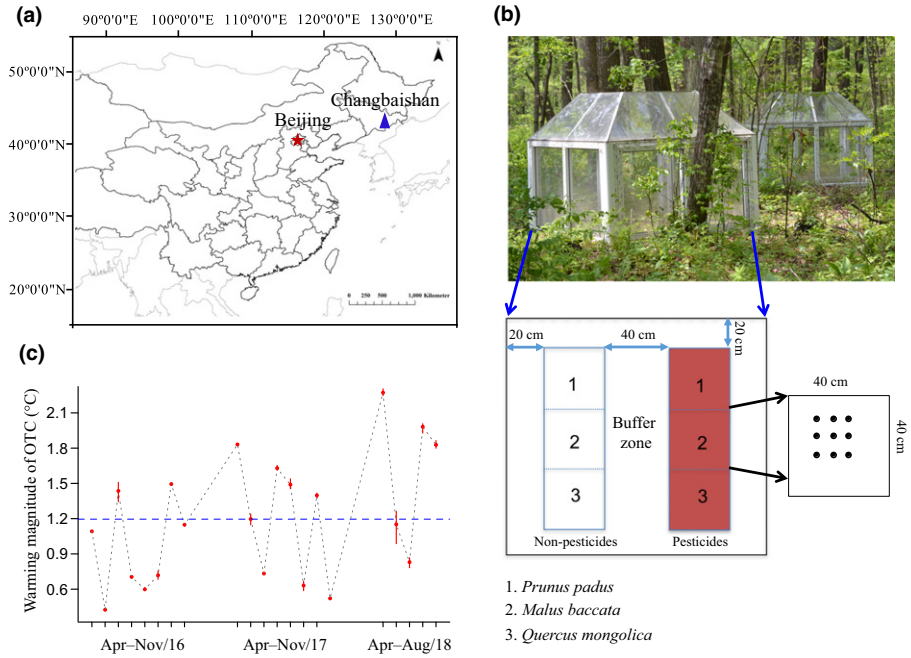

Fig. 1 (a) Location of the study site in Changbaishan Nature Reserve (a temperate forest; 128°5′E, 42°23′N) in northeastern China. (b) Establishment of the experiment in the field, showing open-top chambers (OTCs). For pesticide treatment, two pesticides ('Celest Gold' and 'Ridomil Gold') were administered 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).

reported that the effect of conspecific negative density dependence (CNDD) on seedling survival changed to be positive after 3 yr 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 the weakening 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 bird cherry or hackberry, is native to northern Asia and northern Europe and grows up to *c.* 16 m tall. The *Prunus* genus is widely known as being 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 the Materials and Methods section; Fig. 1b). The experiment involved three factors: warming (two levels of treatment: OTC warming vs control), distance to parent trees (three distances: 1, 10 and 20 m away from a *P. padus* parent tree) and pesticide treatment (watering with pesticides vs without pesticides). For the purpose of comparison and to test 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 3 yr (June 2016 to September 2018). Seedling survival was assessed every 2 wk in the first 2 yr and every 6 wk 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 was 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 reassessed every 5 yr (Supporting Information 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 *c.* 1 ha. In September 2015, three 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 (nine OTCs in total; Fig. 1b). Beside each OTC, a 160 × 160 cm control plot was also established. The control plots were fenced by a 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), although being relatively low compared with that of 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. Therefore, 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-column 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. 1(b). One column of three quadrats was randomly selected to receive pesticide treatment and the other three quadrats were treated with the same amount of sterilised 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 2 wk for the first 2 yr and every 6 wk 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 sterilised clean water and 0.50 g m^{-2} liquid Celest Gold diluted in 1 l of sterilised clean water. Quadrats without pesticide treatment were sprayed with the same amount of sterilised clean water. Seedling mortality was recorded whenever pesticides or watering was applied.

In early June 2016, 2-wk-old seedlings, germinated in sterilised sands in glasshouse 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 nine 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 Thematic Mapper (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 4 h, 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 2 yr. In the third year (2018), soil temperature and moisture measurement were stopped at the end of August (the field experiment ended on 1 September 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 the 3-yr 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 2 yr (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 throughly 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 sterilised sand in a glasshouse.

The sampled soils were immediately frozen and shipped on dry ice to the laboratory at Magigene Technology Ltd in Guangzhou and were stored at −80°C before DNA extraction. Fungal DNA was extracted from 1 g of soil using the PowerSoil® DNA isolate kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA). The concentration and purity of the extracted DNA samples were measured using a NanoDrop One spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Internal transcribed

spacer (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 TRIMMOMATIC (Bolger *et al.*, 2014), paired-end clean reads assembly using FLASH (Magoč & Salzberg, 2011), and raw tags quality control using 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% was recognised 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 8438 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 list 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 publications (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 plants 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 survival 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 3-yr experiment period (June 2016 to September 2018; Table S4), yielding more powerful data for statistical inferences. We first estimated and tested the difference in survival for *P. padus* seedlings under different treatments (Fig. 2). Because the survival times involved censored observations, that is those seedlings that were still alive at the time of observation, the product limit estimate (Kaplan–Meier estimate) of survival function should be used (Lawless, 2002). Survival curves were estimated using the R function *survfit* from the SURVIVAL package (http://survival.r-forge.r-project.org/). Survival curves were also estimated for the nontarget 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 years 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 SURVIVAL package (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 years 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 generalised 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

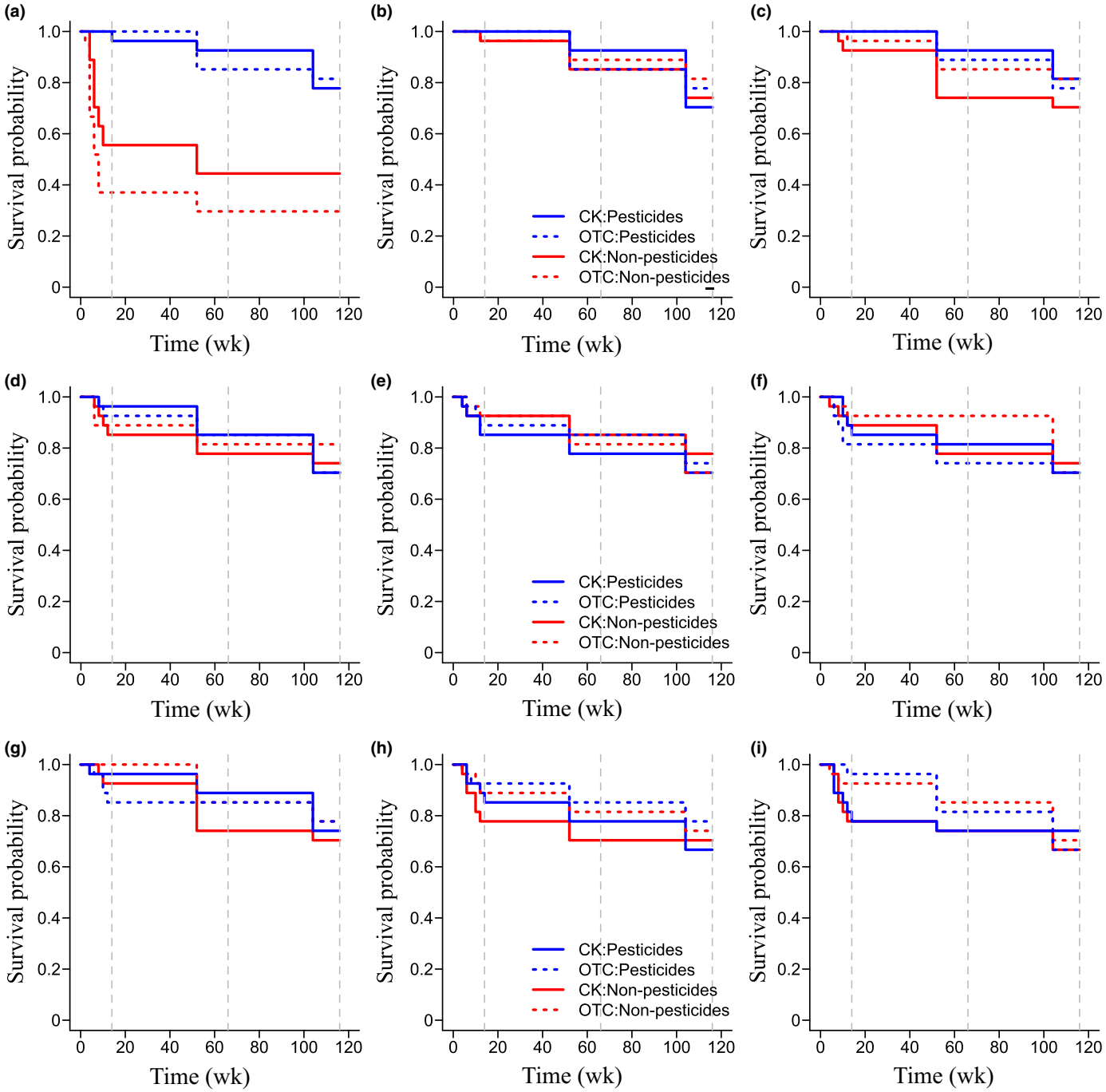

Fig. 2 Survival of seedlings. Kaplan–Meier survival curves for *Prunus padus* seedlings (top row), *Malus baccata* seedlings (middle row) and *Quercus mongolica* seedlings (bottom row). The curves represent four treatment combinations of pesticide and warming at 1 m distance (a, d, g), 10 m distance (b, e, h), and 20 m distance (c, f, i) from the three adult trees of *P. padus* for the 3-yr 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 wk (i.e. the end of the growing season of the first, second and third years). CK, control.

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 neighbourhood not greater than 10 m away from adults (Liu *et al.*, 2012; Murphy *et al.*, 2017), we modelled 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 DBH = 6.2 cm as the threshold (the third quartile of its DBH size). For other (nonfocal) species, DBH = 10 cm was used as the threshold to categorise 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 criticised in previous publications (Detto *et al.*, 2019), we reanalysed 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 survival

The Kaplan–Meier survival 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 survival 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 survival of *P. padus* seedlings were detected at 10 or 20 m distance (at least $P > 0.12$; Fig. 2b,c). By 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 PH models or an accelerated time model for seedling mortality in the plots at 1 m distance around *P. padus* parent trees, the hazard ratios (HR) of *P. padus* seedling mortality under warming were significantly larger than 1 (HR = 1.85 after 1 yr, $P = 1e^{-9}$, Fig. 3a; HR = 1.70 after 2 yr, $P < 2e^{-16}$, Fig. 3b; HR = 1.70 after 3 yr, $P = 3e^{-15}$, Fig. 3c). Consistent with the survival 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

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 the *P. padus* parent trees (Table 1; Fig. S2), supporting the JC hypothesis that pathogens tended 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 vs −11.0), although distance effects on both groups of pathogens were highly significant (Table 1).

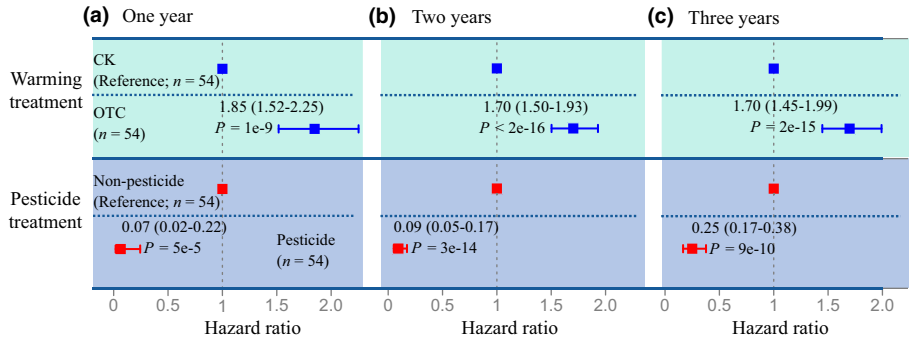

Fig. 3 Hazard ratios (relative risk) of *Prunus padus* seedling mortality. The effects of warming and pesticide treatment on seedling mortality risk based on hazard models for modelling seedling survival time up to the end of the first year (a), the second year (b), and the third year (c) of the experiment. (a) and (b) were modelled using Cox proportional hazard models, while (c) was modelled by an accelerated time model (Weibull regression). A treatment and its control (CK) were included in each same coloured box. A hazard ratio larger than 1 indicates an increased hazard of seedling mortality under a treatment vs control (i.e. the baseline reference). *n*, number of seedlings per treatment.

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

	255 FUNGuild classified pathogens			101 specified *Prunus* pathogens		
Effects	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	$<2e^{-16}$

Coef.: The model coefficients measure the effect size of the treatments. The random effects control for the variation associated with the three adult trees and its variance = 0.05 and 0.06, respectively.

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 confirmed 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 presented in Fig. 4 further showed 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 a 1% increase in the relative abundance of the *Prunus*-specific pathogens (Fig. 4b) but only a 12.8% increase in seedling mortality for a 1% increase in the relative abundance of the 255 FUNGuild classified pathogens (Fig. 4a). By contrast, the mortality HRs for the seedlings of the other two nonfocal species were not significantly larger than 1 over the 3 yr of the 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 the understanding of effects of climate change on tree diversity. We showed that soil-

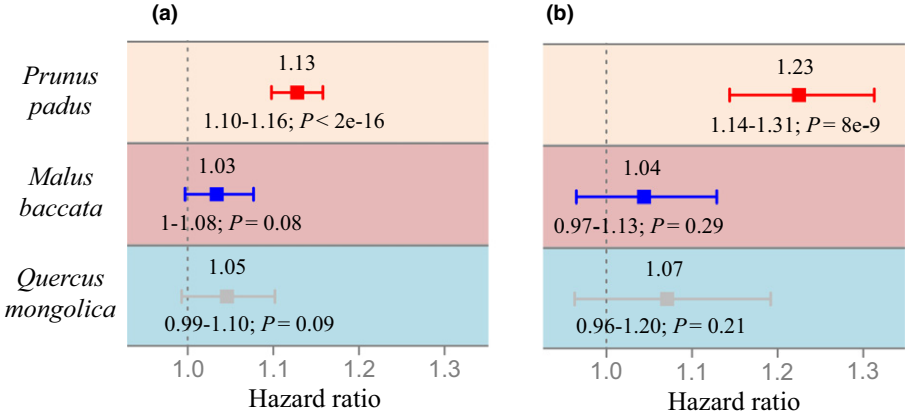

Fig. 4 Hazard ratios (relative risk) estimated from Cox proportional hazard (PH) models for *Prunus padus*, *Malus baccata* and *Quercus mongolica* seedling mortality associated with the relative abundance of plant-pathogenic fungi. (a) For 255 FUNGuild classified pathogenic open-top chambers (OTCs). (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 3-yr field experiment.

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, therefore 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 the HR for *P. padus* seedlings (Fig. 4). For oomycete pathogens, they were suppressed by pesticide treatment but could not be analysed 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). All 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, for example *P. serotine* and *P. virginiana* (Packer & Clay, 2000; Reinhart & Clay, 2009; Bennett *et al.*, 2017), recruitment of *P. padus* seedlings is inhibited by soil pathogens. If the JC effect indeed regulates *P. padus* population as our experiment showed, we should be able to detect the 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 inhibited 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 is likely to 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.*, 2014), 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 has also been shown to alter plant–microbe interactions in natural ecosystems (Rudgers *et al.*, 2014; Hutchins *et al.*, 2019; Liu & He, 2019; Liu *et al.*, 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 attained a maximum *c.* 19°C in August (Fig. S1), well below the optimum temperature (20–25°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 is likely to explain the contrasting results of our study compared with those of Bachelot *et al.* (2020), who found that warming weakened plant–soil feedback. In their study, an increase of 4°C made temperature 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 FUNGuild database (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). For the pathogenic fungi classified by the FUNGuild database, we went further to identify, from independent published sources, for 101 OTUs that had been confirmed as virulent to *Prunus* species. This latter group showed a stronger distance decay in their relative abundance with respect to *P. padus* adult trees compared with 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 lent 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 (Figs 2a, 4).

Despite the evidence for an effect of warming on the JC effect, some caveats are in place. Our sequencing method could suffer similar problems as those summarised by Taylor *et al.* (2016), for example 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 the abundance of fungi using 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 to test 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, for example 5 m away from parent trees, could also be used. Such distances would ensure that seedlings have reduced competition from parent trees compared with those at 1 m distance, but are still subjected to the effect of host-specific pathogens compared with 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 nonfocal species did not, is strong evidence indicating that *P. padus*-specific pathogens are at work.

Our study highlighted the importance of mechanistic understanding of climate warming effect on species diversity, to complement the many studies that have focused 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. It is worth noting however 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. There is a pressing need to expand the current study to other species, climatic zones and global change factors in light of the latest UN emission gap report (https://www.unenvironment.org/resources/emissions-gap-report-2019).

Acknowledgements

We thank Ji Ye, Fei Lin and Zhenshan Li for their assistance in the field. This study was supported by East China Normal University to the ECNU-Alberta Joint Laboratory for Biodiversity Study, the National Natural Science Foundation of China (31670531 to YL), and the Natural Sciences and Engineering Research Council of Canada (to FH).

Author contributions

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

ORCID

Fangliang He https://orcid.org/0000-0003-0774-4849
Yu Liu https://orcid.org/0000-0001-9869-2735

References

Agrios GN. 2005. *Plant pathology*. San Diego, CA, USA: Elsevier Academic Press.

Augspurger CK. 1983. Seed dispersal by the tropical tree, *Platypodium elegans*, and the escape of its seedling from fungal pathogens. *Journal of Ecology* **71**: 759–771.

Augspurger CK. 1984. Seedling survival of tropical tree species: interactions of dispersal distance, light gaps, and pathogens. *Ecology* **65**: 1705–1712.

Bachelot B, Alonso-Rodríguez AM, Aldrich-Wolfe L, Cavaleri MA, Reed SC, Wood TE. 2020. Altered climate leads to positive density-dependent feedbacks in a tropical wet forest. *Global Change Biology* **26**: 3417–3428.

Bagchi R, Gallery RE, Gripenberg S, Gurr SJ, Narayan L, Addis CE, Freckleton RP, Lewis OT. 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. *Nature* **506**: 85–88.

Barford E. 2013. Crop pests advancing with global warming. *Nature* **3**: 985–988.

Bebber DP, Holmes T, Gurr SJ. 2014. The global spread of crop pests and pathogens. *Global Ecology and Biogeography* **23**: 1398–1407.

Bebber DP, Ramotowski MAT, Gurr SJ. 2013. Crop pests and pathogens move polewards in a warming world. *Nature Climate Change* **3**: 985–988.

Bell T, Freckleton RP, Lewis OT. 2006. Plant pathogens drive density-dependent seedling mortality in a tropical tree. *Ecology Letters* **9**: 569–574.

Bennett JA, Maherali H, Reinhart KO, Lekberg Y, Hart MM, Klironomos J. 2017. Plant–soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science* **355**: 181–184.

Bever JD, Mangan S, Alexander HM. 2015. Maintenance of plant species diversity by pathogens. *Annual Review of Ecology, Evolution, and Systematics* **46**: 305–325.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114–2120.

Chakraborty S. 2013. Migrate or evolve: options for plant pathogens under climate change. *Global Change Biology* **19**: 1985–2000.

Chakraborty S, Tiedemann AV, Teng PS. 2000. Climate change: potential impact on plant diseases. *Environmental Pollution* **108**: 317–326.

Chen L, Swenson NG, Ji N, Mi X, Ren H, Guo L, Ma K. 2019. Differential soil fungus accumulation and density dependence of trees in a subtropical forest. *Science* **366**: 124–128.

Connell JH. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: Boer PJD, Gradwell GR, eds. *Dynamics of populations*. Wageningen, the Netherlands: Center for Agriculture Publishing and Documentation, 298–312.

Day NJ, Dunfield KE, Johnstone JF, Mack MC, Turetsky MR, Walker XJ, White AL, Baltzer JL. 2019. Wildfire severity reduces richness and alters composition of soil fungal communities in boreal forests of western Canada. *Global Change Biology* **25**: 2310–2324.

De Frenne P, Brunet J, Shevtsova A, Kolb A, Graae Bj, Chabrerie O, Cousins SA, Decocq G, De Schrijver AN, Diekmann M *et al.* 2011. Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. *Global Change Biology* **17**: 3240–3253.

Delgado-Baquerizo M, Guerra CA, Cano-Díaz C, Egidi E, Wang J-T, Eisenhauer N, Singh BK, Maestre FT. 2020. The proportion of soil-borne pathogens increases with warming at the global scale. *Nature Climate Change* **10**: 550–554.

Detto M, Visser MD, Wright SJ, Pacala SW. 2019. Bias in the detection of negative density dependence in plant communities. *Ecology Letters* **22**: 1923–1939.

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**: 2460–2461.

Francl LJ. 2001. The disease triangle: A plant pathological paradigm revisited. *The Plant Health Instructor*. doi: 10.1094/PHI-T-2001-0517-01.

Freckleton RP, Lewis OT. 2006. Pathogens, density dependence and the coexistence of tropical trees. *Proceedings of the Royal Society B: Biological Sciences* **273**: 2909–2916.

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD. 2002. Climate warming and disease risks for terrestrial and marine biota. *Science* **296**: 2158–2162.

Hille Ris Lambers J, Clark JS, Beckage B. 2002. Density-dependent mortality and the latitudinal gradient in species diversity. *Nature* **417**: 732–735.

Hollister RD, Webber PJ. 2000. Biotic validation of small open-top chambers in a tundra ecosystem. *Global Change Biology* **6**: 835–845.

Hutchins DA, Jansson JK, Remais JV, Rich VI, Singh BK, Trivedi P. 2019. Climate change microbiology — problems and perspectives. *Nature Review Microbiology* **17**: 391–396.

IPCC. 2013. *Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change*. Cambridge, UK.

Janzen DH. 1970. Herbivores and the number of tree species in tropical forests. *American Naturalist* **104**: 501–528.

Jia S, Wang X, Yuan Z, Lin F, Ye J, Lin G, Hao Z, Bagchi R. 2020. Tree species traits affect which natural enemies drive the Janzen-Connell effect in a temperate forest. *Nature Communications* **11**: 286.

Johnson DJ, Beaulieu WT, Bever JD, Clay K. 2012. Conspecific negative density dependence and forest diversity. *Science* **336**: 904–907.

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM *et al.* 2013. Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* **22**: 5271–5277.

LaManna JA, Mangan SA, Alonso A, Bourg NA, Brockelman WY, Bunyavejchewin S, Chang LW, Chiang JM, Chuyong GB, Clay K *et al.* 2017. Plant diversity increases with the strength of negative density dependence at the global scale. *Science* **356**: 1389–1392.

LaManna JA, Mangan SA, Myers JA. 2021. Conspecific negative density dependence and why its study should not be abandoned. *Ecosphere* **12**: e03322.

Launay M, Caubel J, Bourgeois G, Huard F, Garcia de Cortazar-Atauri I, Bancal M-O, Brisson N. 2014. Climatic indicators for crop infection risk: Application to climate change impacts on five major foliar fungal diseases in Northern France. *Agriculture, Ecosystems & Environment* **197**: 147–158.

Lawless JF. 2002. *Statistical models and methods for lifetime data*. New York, NY, USA: John Wiley & Sons.

Leff JW, Bardgett RD, Wilkinson A, Jackson BG, Pritchard WJ, De Long JR, Oakley S, Mason KE, Ostle NJ, Johnson D *et al.* 2018. Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. *ISME Journal* **12**: 1794–1805.

Legeay J, Husson C, Cordier T, Vacher C, Marcais B, Buée M. 2019. Comparison and validation of Oomycetes metabarcoding primers for *Phytophthora* high throughput sequencing. *Journal of Plant Pathology* **101**: 743–748.

Levi T, Barfield M, Barrantes S, Sullivan C, Holt RD, Terborgh J. 2019. Tropical forests can maintain hyperdiversity because of enemies. *Proceedings of the National Academy of Sciences, USA* **116**: 581–586.

Li Y, Sun D, Li D, Xu Z, Zhao C, Lin H, Liu Q. 2015. Effects of warming on ectomycorrhizal colonization and nitrogen nutrition of *Picea asperata* seedlings grown in two contrasting forest ecosystems. *Scientific Reports* **5**: 17546.

Liu X, Ma Z, Cadotte MW, Chen F, He JS, Zhou S. 2019. Warming affects foliar fungal diseases more than precipitation in a Tibetan alpine meadow. *New Phytologist* **221**: 1574–1584.

Liu Y, Fang S, Chesson P, He F. 2015. The effect of soil-borne pathogens depends on the abundance of host tree species. *Nature Communications* **6**: 10017.

Liu Y, He F. 2019. Incorporating the disease triangle framework for testing the effect of soil-borne pathogens on tree species diversity. *Functional Ecology* **33**: 1211–1222.

Liu Y, Reich PB, Li G, Sun S. 2011. Shifting phenology and abundance under experimental warming alters trophic relationships and plant reproductive capacity. *Ecology* **92**: 1201–1207.

Liu Y, Yu S, Xie ZP, Staehelin C. 2012. Analysis of a negative plant-soil feedback in a subtropical monsoon forest. *Journal of Ecology* **100**: 1019–1028.

Magoč T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **27**: 2957–2963.

Mangan SA, Schnitzer SA, Herre EA, Mack KM, Valencia MC, Sanchez EI, Bever JD. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* **466**: 752–755.

Milici VR, Dalui D, Mickley JG, Bagchi R, Fridley J. 2020. Responses of plant–pathogen interactions to precipitation: Implications for tropical tree richness in a changing world. *Journal of Ecology* **108**: 1800–1809.

Mommer L, Cotton TEA, Raaijmakers JM, Termorshuizen AJ, van Ruijven J, Hendriks M, van Rijssel SQ, van de Mortel JE, van der Paauw JW, Schijlen EGWM *et al.* 2018. Lost in diversity: the interactions between soil-borne fungi, biodiversity and plant productivity. *New Phytologist* **218**: 542–553.

Murphy SJ, Wiegand T, Comita LS. 2017. Distance-dependent seedling mortality and long-term spacing dynamics in a neotropical forest community. *Ecology Letters* **20**: 1469–1478.

Newsham KK, Hopkins DW, Carvalhais LC, Fretwell PT, Rushton SP, O'Donnell AG, Dennis PG. 2016. Relationship between soil fungal diversity and temperature in the maritime Antarctic. *Nature Climate Change* **6**: 182–185.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Nilsson RH, Larsson K-H, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Glöckner FO, Tedersoo L *et al.* 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research* **47**: D259–D264.

Olofsson J, Ericson L, Torp M, Stark S, Baxter R. 2011. Carbon balance of Arctic tundra under increased snow cover mediated by a plant pathogen. *Nature Climate Change* **1**: 220–223.

Packer A, Clay K. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. *Nature* **404**: 278–281.

Parmesan C, Yohe G. 2004. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**: 37–42.

Petermann JS, Fergus AJF, Turnbull LA, Schmid B. 2008. Janzen–Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* **89**: 2399–2406.

van der Putten WH, Bradford MA, Brinkman EP, van de Voorde TFJ, Veen GF. 2016. Where, when and how plant–soil feedback matters in a changing world. *Functional Ecology* **30**: 1109–1121.

Reinhart KO, Clay K. 2009. Spatial variation in soil-borne disease dynamics of a temperate tree, *Prunus serotina*. *Ecology* **90**: 2984–2993.

Rudgers JA, Kivlin SN, Whitney KD, Price MV, Waser NM, Harte J. 2014. Responses of high-altitude graminoids and soil fungi to 20 years of experimental warming. *Ecology* **95**: 1918–1928.

Scherm H, van Bruggen AHC. 1994. Global warming and nonlinear growth: how important are changes in average temperature? *Phytopathology* **84**: 1380–13384.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ *et al.* 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**: 7537–7541.

Semchenko M, Leff JW, Lozano YM, Saar S, Davison J, Wilkinson A, Jackson BG, Pritchard WJ, De Long JR, Oakley S *et al.* 2018. Fungal diversity regulates plant–soil feedbacks in temperate grassland. *Science. Advances* **4**: eaau4578.

Sheik CS, Beasley WH, Elshahed MS, Zhou X, Luo Y, Krumholz LR. 2011. Effect of warming and drought on grassland microbial communities. *ISME Journal* **5**: 1692–1700.

Siebold M, von Tiedemann A. 2013. Effects of experimental warming on fungal disease progress in oilseed rape. *Global Change Biology* **19**: 1736–1747.

Spear ER, Coley PD, Kursar TA, Thrall P. 2015. Do pathogens limit the distributions of tropical trees across a rainfall gradient? *Journal of Ecology* **103**: 165–174.

Sturrock RN, Frankel SJ, Brown AV, Hennon PE, Kliejunas JT, Lewis KJ, Worrall JJ, Woods AJ. 2011. Climate change and forest diseases. *Plant Pathology* **60**: 133–149.

Swinfield T, Lewis OT, Bagchi R, Freckleton RP. 2012. Consequences of changing rainfall for fungal pathogen-induced mortality in tropical tree seedlings. *Ecology and Evolution* **2**: 1408–1413.

Tapsoba H, Wilson JP. 1997. Effects of temperature and light on germination of urediniospores of the pearl millet rust pathogen, *Puccinia substriata* var. *indica*. *Plant Disease* **81**: 1049–1052.

Taylor DL, Walters WA, Lennon NJ, Bochicchio J, Krohn A, Caporaso JG, Pennanen T. 2016. Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for Illumina amplicon sequencing. *Applied and Environmental Microbiology* **82**: 7217–7226.

Tedersoo L, Drenkhan R, Anslan S, Morales-Rodriguez C, Cleary M. 2019. High-throughput identification and diagnostics of pathogens and pests: overview and practical recommendations. *Molecular Ecology Resources* **19**: 47–76.

Veresoglou SD, Barto EK, Menexes G, Rillig MC. 2013. Fertilization affects severity of disease caused by fungal plant pathogens. *Plant Pathology* **62**: 961–969.

Wang X, Wiegand T, Hao Z, Li B, Ye J, Lin F. 2010. Species associations in an old-growth temperate forest in north-eastern China. *Journal of Ecology* **98**: 674–686.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

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.

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 generalised linear mixed model for modelling responses of relative abundance of plant-pathogenic fungi to experimental treatments.

Table S1 All OTUs for 36 soil samples.

Table S2 Fungal OTUs for 36 soil samples.

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

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

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.