2005

Soil feedback and pathogen activity in *Prunus serotina* throughout its native range

Kurt O. Reinhart
Indiana University, kureinha@indiana.edu

Alejandro A. Royo
USDA NERS, Forestry Sciences Laboratory

Wim H. Van Der Putten
Netherlands Institute of Ecology, Centre for Terrestrial Ecology

Keith Clay
Indiana University

Journal of Ecology 2005
93, 890–898

Soil feedback and pathogen activity in *Prunus serotina* throughout its native range

KURT O. REINHART, ALEJANDRO A. ROYO*, WIM H. VAN DER PUTTEN† and KEITH CLAY

*Department of Biology, Indiana University, Bloomington, Indiana, 47405–3700, USA, *USDA NERS, Forestry Sciences Laboratory, Irvine, PA 16329, USA, and †Netherlands Institute of Ecology, Centre for Terrestrial Ecology, Multitrophic Interactions Department, PO Box 40, 6666 ZG Heteren, the Netherlands*

Summary

1 Oomycete soil pathogens are known to have a negative effect on *Prunus serotina* seedling establishment and to promote tree diversity in a deciduous forest in Indiana, USA. Here, we investigate whether negative feedbacks operate widely in its native range in eastern USA.

2 In laboratory experiments, soil sterilization was used to test the net effect of soil biota (pathogens and mutualists) and fungicide treatments to test the effects of soil pathogens (oomycetes) on survival of *P. serotina* seedlings in soils from 22 *P. serotina* populations throughout the eastern USA.

3 In soil associated with *P. serotina* trees, there was a significant positive effect of both soil sterilization and fungicide on seedling survival. The two effects were positively correlated, suggesting that oomycetes were responsible for the observed mortality of seedlings in untreated soils relative to sterilized soils.

4 We determined the host-specificity of these interactions by comparing the effects of the soil biota associated with conspecific and heterospecific trees. There was no interaction between the effects of soil origin and soil sterilization, or of soil origin and fungicide, on seedling survival, although an effect of soil origin on the relative oomycete effect suggested that soil pathogens associated with conspecifics had a more negative influence than those from heterospecifics.

5 Fungicide treatment decreased pre-emergence mortality of *P. serotina* seedlings at two of three field sites in the northern USA.

6 The overall consistency between the laboratory experiments and the field experiment strongly suggests that oomycete soil pathogens have a negative effect on the survival of *P. serotina* seedlings throughout its native range in the eastern USA.

7 Soil-borne pathogens therefore appear to regulate the densities of a common tree species (*P. serotina*) at larger geographical scales than previously described, providing additional evidence of the important role that soil biota play in regulating plant populations and structuring plant communities.

Key-words: black cherry, invasive species, macroecology, oomycetes, plant disease, plant–soil biota interactions, *Prunus serotina*, *Pythium*, soil-borne pathogens

Journal of Ecology (2005) **93**, 890–898
doi: 10.1111/j.1365-2745.2005.01028.x

Introduction

The diversity of pathogens, parasites, symbionts and decomposers in the soil has been shown to affect successional dynamics, diversity and productivity of terrestrial plant communities (Wardle 2002). Several studies describing the influence of plants on soil communities report a negative feedback that enhances the rate of succession (Van der Putten *et al.* 1993), affects tree establishment into certain habitats (O'Hanlon-Manners & Kotanen 2004), influences plant abundance

Correspondence: Kurt O. Reinhart (fax 812-855-6705; e-mail kureinha@indiana.edu).

(Klironomos 2002) and affects species diversity (Mills & Bever 1998; Bever 2003). A net negative effect of the soil biota on plant growth, survival or fitness has been described for numerous species in grasslands (Hartnett & Wilson 1999; Olff *et al*. 2000; Klironomos 2002; Bever 2003) and dunes (Van der Putten *et al*. 1993), as well as in tropical (Augspurger 1984; Hood *et al*. 2004) and temperate forests (Packer & Clay 2000, 2002, 2004) and agricultural systems (Zhang & Yang 2000). These interactions serve as a form of density-dependent regulation and play an important role in maintaining the diversity of terrestrial plant communities (Bever 2003). Extrapolation of the numerous results is, however, limited to relatively small geographical scales because the experiments were not designed to characterize the plant–soil biota interactions throughout the entire range of individual plant species.

A regional perspective (Brown 1995) is necessary to determine the generality and geographical extent of such feedbacks and their consequences in structuring plant communities. Previous research from a mesic deciduous forest (Bloomington, Indiana, USA) indicates that *Prunus serotina* (black cherry) trees accumulate soil-borne pathogens (particularly from the oomycete genus *Pythium*), which have density- and distance-dependent effects on seedling mortality and growth of seedlings and saplings of conspecifics (Packer & Clay 2000, 2002, 2004). *Pythium* spp. are known to cause damping-off disease, which may result in pre- and post-emergence mortality and, if not fatal, may reduce the growth of infected plants (Martin & Loper 1999). Although *P. serotina* is broadly distributed in North America (Little 1977), little is known about the plant–soil biota interactions for this species outside of populations in Bloomington, Indiana, USA.

Large-scale geographical investigations are important because *P. serotina* is invasive in north-western Europe and the release of plants from their native soil pathogens has been suggested to enhance invasiveness (Klironomos 2002; Reinhart *et al*. 2003; Callaway *et al*. 2004; Knevel *et al*. 2004; Reinhart & Callaway 2004). Bioassays with soil from a site in the Netherlands showed a net positive plant–soil biota interaction for *P. serotina* (Reinhart *et al*. 2003), suggesting that invasive plants may experience a net benefit from soil communities in their non-native range. To clarify whether negative responses can be generalized across large spatial scales and whether the interactions are fundamentally different in native and non-native ranges, we investigated the effects of soil biota on *P. serotina* throughout the eastern USA. Only if negative plant–soil biota interactions are prevalent across the native range could invasive success in Europe be attributed to escape from natural enemies.

Negative effects associated with the soil community (e.g. pathogens and mutualists) and soil-borne oomycetes (e.g. *Pythium* and *Phytophthora*) have been described for *P. serotina* in Indiana (Packer & Clay 2000). We used manipulative laboratory experiments to test the net effect of soil biota (via soil sterilization) and oomycete soil pathogens (via selective fungicide) in other parts of the range of *P. serotina* on seedling survival. A field experiment was also used to test the effect of oomycetes on the pre-emergence mortality of seedlings in natural populations. We determined the host-specificity of any effects by comparing the effects of soil biota and soil pathogens originating from soil near conspecific vs. heterospecific trees. Other studies have shown that host-specific effects often develop in association with plant species, which help to maintain plant diversity (e.g. Van der Putten *et al*. 1993; Bever 1994; Packer & Clay 2000; Bever 2002; Klironomos 2002). Understanding the geographical and spatial variation in plant–soil biota interactions and their degree of host-specificity may help to explain large-scale patterns of abundance, dominance and range limits of plant species (Reynolds *et al*. 2003).

Methods

STUDY SITES

The contiguous range of *Prunus serotina* is from Nova Scotia and New Brunswick west to Southern Quebec and Ontario into Michigan and eastern Minnesota, south to Iowa, eastern Nebraska, Oklahoma and Texas, and then east to central Florida (Little 1977). Two disjunct subspecies, often with isolated populations, are located in portions of Texas west to Arizona and south into Guatemala (Little 1977). For the laboratory experiments, soil was collected adjacent to individual trees of *P. serotina* from 22 populations at eight sites located throughout the eastern USA.

The populations were associated with varying environments, soils and plant communities. Species inventories, personal accounts and other factors, such as accessibility, were used to identify sites containing *P. serotina* and the number of populations selected per site (one to four, Table 1) reflects the size of the site, the prevalence of *P. serotina* and the heterogeneity of the site. *Prunus serotina* occurred on soils with varying amounts of sand, organic matter and clay and in varied plant communities (e.g. oak, pine and mesic deciduous forests) typical of this species (Marquis 1990). The average minimum distance between populations within a site was 7.3 ± 3.3 km (± SE) (Table 1). The assumption that *P. serotina* populations were independent within a site was verified by determining that there was as much variation in the seedling survival data within a site as between sites (data not shown).

SOIL BIOTA EXPERIMENT

The soil biota and soil pathogen experiments were performed simultaneously and identical soil types (i.e. background soil and sterilized and non-sterile treatment soils) were used, except that pathogens were also selectively removed by application of fungicide (see

Table 1 Latitude and longitude coordinates of soil collection sites, number of populations per site, minimum distance between populations at individual sites, and seed source used to plant laboratory experiments

Sites	ANF	Blm	GSMNP	HNF	IDNL	MCNP	SFHPSP	TSP
Latitude	41°45′ N	39°01′ N	35°42′ N	31°15′ N	41°37′ N	37°11′ N	29°42′ N	30°34′ N
Longitude	079°11′ W	086°18′ W	083°21′ W	091°00′ W	087°05′ W	086°04′ W	082°27′ W	084°56′ W
Populations site^{-1}	3	3	4	3	3	3	1	2
Distance (km)	24.3	1.5	3.3	13.3	6.2	1.9	–	0.8
Seed source	PA	Blm	PA	MI	MI	Blm	MI	MI

Site abbreviations: Allegheny National Forest (ANF), Pennsylvania; Bloomington (Blm), Indiana; Homochitto National Forest (HNF), Mississippi; The Great Smoky Mountains National Park (GSMNP), Tennessee; Indiana Dunes National Lakeshore (IDNL), Indiana; Mammoth Cave National Park (MCNP), Kentucky; San Felasco Hammock Preserve State Park (SFHPSP), Florida; and Torreya State Park (TSP), Florida. Seed source abbreviations: PA, Pennsylvania; Blm, Bloomington, Indiana; and MI, Michigan.

below). To test the net effect of the soil biota (i.e. pathogens and mutualists) on *Prunus serotina*, we compared the effects of non-sterile vs. sterilized soils on the survival of *P. serotina* seedlings, using soil originating near conspecific trees. Comparison with the effects of soil originating near heterospecific trees was used to test for host-specificity, because unique soil communities are known to develop in association with neighbouring species (e.g. Mills & Bever 1998; Klironomos 2002; Bever 2003). For all populations, we selected three mid- or overstorey *Prunus serotina* trees (two for each of the four Great Smoky Mountains National Park populations, Table 1): average diameter at breast height was 40 cm (range 13.3–69.9 cm). We haphazardly selected three neighbouring non-*Prunus* trees per population: these were typically ≥ 20 m away from the nearest *P. serotina*, except at Allegheny National Forest (ANF), where this species is often dominant. No single species was a common heterospecific in all populations and species sampled in the diverse forest communities often varied within and between populations, but included *Acer* spp., *Fraxinus* spp., *Pinus* spp. and *Quercus* spp.

Soil samples were collected from six to eight points around each conspecific and heterospecific tree. Soil cores (*c*. 2.5 cm diameter) were collected from a depth of 0–10 cm and at a distance of 1.5 m away from the focal tree. Soil cores for individual trees were aggregated into one bulk sample of *c*. 1 L soil tree^{-1}. The soil probe was flame sterilized before sampling around each individual tree to ensure independence of samples from different trees. Additionally, several litres of soil (0–10 cm depth) were collected with a hand trowel from haphazardly selected locations within each population. This soil was autoclaved and is referred to as 'background soil' (representative of the soil from each *P. serotina* population). This provided a sterilized medium, consistent with the soil texture and fertility in the field, in which seedlings and soil biota could interact.

Soil was collected in the winter (November 2003 to January 2004) to control for phenological and environmental differences between sites. The soil biota was assumed to be present as resting states (e.g. spores) to endure the winter, and therefore more likely to survive transport and cold storage than summer samples. Sampling was started in the higher latitudes and the southern populations were sampled last. Following collection, soils were transported in a cooler to Indiana University and stored at 4 °C. During transit, efforts were made to maintain the soil at a constant temperature and avoid temperature extremes. All of the field soil was prepared by dicing the roots and crumbling the soil until it passed through a 1-cm^2 mesh sieve. To avoid cross-contamination and transfer of soil biota between experimental units, all tools (e.g. sieves), materials and surfaces coming in contact with non-sterilized soil were either autoclaved for 20–180 minutes (depending on quantity of material), flame sterilized, or had their surfaces sprayed or material soaked in ≥ 10% bleach solution.

Prunus serotina seeds were collected in Bloomington, Indiana. Many sites contained such low densities of *P. serotina* trees that they were not likely to yield enough seeds for our experiment. Therefore, seed was also purchased from Louisiana (Louisiana Forest Seed Co., Lecompte, LA, USA), Michigan and Pennsylvania sources (Sheffield's Seed Co., Locke, NY, USA). Seeds were surface sterilized (solution of 10% bleach for 10 minutes) and then thoroughly rinsed with deonized water, before soaking in water for 24 hours and then cold stratification until they germinated. Germinated seeds had an exposed radicle (2–30 mm long) and no exposed shoots, apart from those from Louisiana, which had a small radicle (2–5 mm) and etiolated shoots.

For the soil biota experiment, 120 mL of autoclaved background soil from each population was added to individual plastic pots (180 mL capacity) and 50 mL of treatment soil (either sterilized or non-sterile) was then spread on the surface (n = 2–3 pots × treatment^{-1} × population^{-1}). Thus, the background soil represented 71% of the total soil in the pot and helped minimize any fertility differences between the treatment soils (Troelstra *et al*. 2001).

Three recently germinated seedlings were then added to each pot and covered with pasteurized potting soil from the Indiana University glasshouse. The potting soil helped create a common covering for all seedlings and homogenized surface evaporation rates across

populations. We planted seeds in soils from the same region (Table 1), except that severe transplant shock of Louisiana seedlings (random with respect to soil treatments) meant that soils from the southern sites (Homochitto National Forest, San Felasco Hammock Preserve State Park and Torreya State Park) were replanted with seedlings from Michigan seed some 2 weeks later. Some seedlings in Bloomington (Blm) and Mammoth Cave National Park (MCNP) soil also experienced transplant shock across all soil treatments (unpublished data): dead seedlings were removed and pots were replanted 2 weeks after initial planting. The portion of the experiment representing a single *P. serotina* population was planted over a 12-hour period, but planting of different seed ecotypes was staggered from 26 April to 24 May because of the unpredictable timing of germination and other logistical constraints.

Seedlings were grown on growth carts in a laboratory illuminated with fluorescent lights (12 hours day^{-1}). The controlled environment and the overlap in growing period controlled for differences between the start times of the different portions of the experiment. Pots were watered every 2–3 days and were treated individually to avoid splashing and dispersal of soil microbes between pots. Sixty days after the planting of each experiment, seedling survival was determined.

SOIL-BORNE PATHOGEN EXPERIMENT

Pots were set up as in the soil biota experiment, except that a $2 \times 2 \times 2$ factorial design was used, with soil sterilization, soil origin and fungicide as the treatments. The fungicide Subdue GR (granules, active ingredient metalaxyl) was applied at a rate of *c*. 0.0055 g fungicide pot^{-1} (Syngenta Crop Protection, Inc. Greensboro, NC, USA). We used this fungicide, which is selective for oomycetes and is used to control *Pythium* and *Phytophora* spp. (known to cause root rot and damping-off diseases; Schwinn & Staub 1987; Paul *et al*. 1989; but see Hood *et al*. 2004), although metalaxyl, the active ingredient, actually interferes with fungal development rather than killing the fungi (Schwinn & Staub 1987). The application rate was based on the rate described in the literature (2 g m^{-2}, Paul *et al*. 1989). Seedlings were planted into pots with sterilized or non-sterile field soil inoculum (50 mL), and fungicide or no fungicide was added. The sterilized soil tested whether the fungicide had a direct, chemical, effect, but there was no difference in seedling survival between fungicide treatments (data not shown).

EFFECT OF SOIL-BORNE PATHOGENS IN THE FIELD

To test the effect of soil-borne oomycetes on the survival of *Prunus serotina* seedlings in the field, recently germinated seeds were transplanted near *P. serotina* or non-*Prunus* spp. (n = 3–4 trees population^{-1}) in Blm on 28–29 April 2004 (n = 3 populations), in Indiana Dunes National Lakeshore (IDNL) on 4 May 2004 (n = 4 populations, 3 of the 4 populations are as described in Table 1) and in second growth stands located near the ANF (41°37′ N, 078°43′ W; 41°43′ N, 078°27′ W; and 41°32′ N, 079°10′ W) on 15 May 2004 (n = 3 populations) (land owned by Kane Hardwood, a division of Collins Pine Co.). Other sites were not included because of environmental restrictions regarding the application of fungicide and other logistical constraints. The Indiana sites (Blm and IDNL) were planted with seed collected in Blm, and ANF with seed from Pennsylvania. Two plots were established near each tree (1 m from focal tree and 0.5 m apart) and each was planted with three seeds with radicles, *c*. 10 cm apart and forming an equilateral triangle. Seeds were covered with pasteurized potting soil from the Indiana University glasshouse. Approximately 0.0125 g of fungicide granules (Subdue GR) were randomly interspersed between the three seeds of one randomly selected plot tree^{-1}. All plots were watered once at the start of the experiment. Seedling emergence and survival was then checked *c*. 45 days after the start of the experiment.

STATISTICAL ANALYSES

For the laboratory experiments, we tested the effect of soil sterilization (i.e. net effect of soil biota) and fungicide (i.e. effect of soil-borne oomycetes) on the survival of seedlings planted in conspecific soil using one-way ANOVA. We determined the host-specificity of these effects by testing the effect of soil treatment (sterilization or fungicide treatments) and soil origin (conspecific vs. heterospecific) on the survival of seedlings using two-way ANOVA, all factors fixed. All analyses were performed with SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). For soil from within an individual population, the percentage survival data for samples taken close to individual trees (n = 2–3 conspecific or heterospecific trees × population^{-1}) were averaged and analysed as a continuous variable. A two-way ANOVA was also used to test the effect of soil origin and sterilization on the Blm populations (n = 3), and these results were compared with the results of a previous study (Packer & Clay 2000). A further two-way ANOVA was used to test the effect of soil origin and fungicide on the IDNL populations (n = 3), and these results were compared with the results from the field experiment.

We calculated the relative soil biota effect and relative oomycete effect for the mean survival in each soil treatment × soil origin × population combination using $(X_c - X_t)/x$, modified relative neighbour effect from Markham & Chanway (1996), where X is the average seedling survival population^{-1} in untreated (c) and treated (t) soil, and x is the higher of X_c and X_t. The relative effect can range from −1 to + 1: negative numbers indicate a positive effect of soil treatment (sterilization or fungicide) on seedling survival relative to untreated soils. We tested the correlation between the relative soil biota and relative oomycete effects for the soil collected

near conspecifics for each population with linear regression. A positive correlation would indicate that the soil biota effect is driven by the effect of the soil pathogens (oomycetes). As described above, a two-way ANOVA was used to determine the host-specificity of the interactions (i.e. a significant interactive effect of soil treatment and soil origin). In addition, we tested the effect of soil origin (conspecific and heterospecific) on the relative soil biota effect and relative oomycete effect with one-way ANOVA to determine the host-specificity of interactions.

For the soil-borne pathogen field experiment, we tested the effect of tree (conspecific vs. heterospecific), fungicide and site on the emergence of *P. serotina* seedlings using a three-way ANOVA with tree (conspecific vs. heterospecific) and fungicide as fixed factors and site as a random factor. For an individual population, the percentage survival data for individual trees (n = 3–4 conspecific or heterospecific trees × population^{-1}) were averaged and analysed as a continuous variable.

Results

In laboratory experiments, we identified a negative effect of soil biota and soil pathogens from throughout the eastern USA on the survival of *Prunus serotina* seedlings. In conspecific soils, sterilizing the soil increased the survival of *P. serotina* seedlings by an average of 31% for 22 populations ($F_{\text{sterilization}} = 5.92$, d.f. = 1,42, $P = 0.019$, Fig. 1a) and application of a selective fungicide increased survival by 27% ($F_{\text{fungicide}} = 4.16$, d.f. = 1,42, $P = 0.048$, Fig. 1a). There was a positive correlation between the relative soil biota effect and relative oomycete effect (ANOVA, $F_{1,21} = 24.1$, $P < 0.0005$, $R^2 = 0.54$, Fig. 2), suggesting that pathogenic oomycetes were responsible for most of the mortality in the soil sterilization treatments.

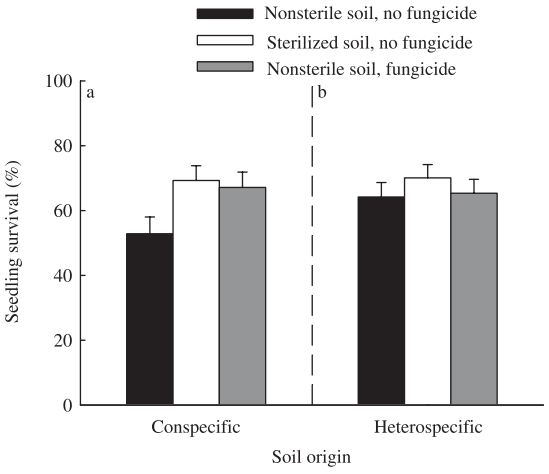

Fig. 1 Laboratory experiments testing the effect of soil treatment (sterilization and fungicide) on the survival of *Prunus serotina* seedlings growing in either conspecific (a) or heterospecific soils (b) collected throughout the eastern USA (n = 22 populations). Bars represent means ± 1 SE.

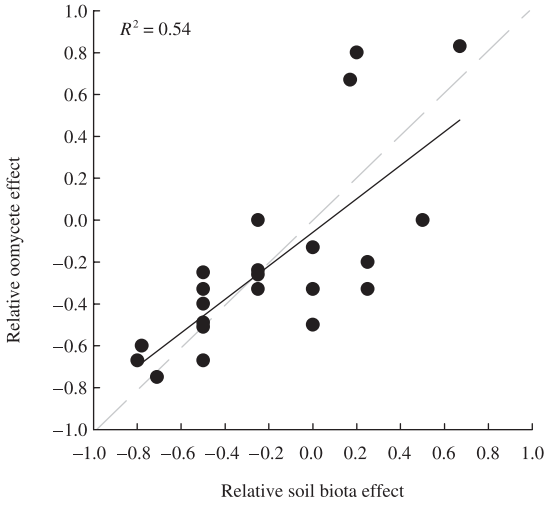

Fig. 2 Correlation between the effect of the soil sterilization (relative soil biota effect) and fungicide (relative oomycete effect) on the survival of *Prunus serotina* seedlings growing in soil collected near conspecific trees growing throughout the eastern USA (n = 22 populations). Data are from the laboratory experiments. Negative relative soil biota effect and relative oomycete effect values indicate a positive effect of soil treatment (sterilization or fungicide, respectively) on seedling survival relative to untreated soils. The dashed line represents a positive 1–1 relationship between the effects.

When heterospecific soils were used, sterilization increased the survival of *P. serotina* seedlings by only 20% ($F_{\text{sterilization}} = 5.27$, d.f. = 1,84, $P = 0.024$) and there was no effect of fungicide ($F_{\text{fungicide}} = 2.05$, d.f. = 1,84, $P = 0.758$) (Fig. 1a,b). Soil origin did not affect seedling survival (soil biota experiment, $F_{\text{origin}} = 0.59$, d.f. = 1,84, $P = 0.446$; soil pathogen experiment, $F_{\text{origin}} = 0.09$, d.f. = 1,84, $P = 0.758$) and there was no interaction between soil origin and soil treatment (soil biota experiment, $F_{\text{sterilization} \times \text{origin}} = 1.04$, d.f. = 1,84, $P = 0.310$; soil pathogen experiment, $F_{\text{fungicide} \times \text{origin}} = 1.68$, d.f. = 1,84, $P = 0.199$) (Fig. 1a,b), suggesting the negative effect of the soil biota and oomycetes on seedling survival was not host-specific (i.e. associated more with conspecifics than heterospecifics).

Although there was no effect of soil origin on the relative soil biota effects ($F_{1,43} = 0.77$, $P = 0.386$, Fig. 3a), there was a marginally significant effect ($0.10 = P > 0.05$) on the relative oomycete effects ($F_{1,43} = 3.88$, $P = 0.055$, Fig. 3b), suggesting the soil pathogens associated with conspecific trees might have a more negative affect on seedling survival than pathogens associated with heterospecifics. Moreover, this effect was statistically significant when the relative oomycete effects were always calculated with X_c, as the denominator, rather than the higher value of X_c and X_t ($F_{1,43} = 5.46$, $P = 0.024$, data not shown).

In the field, there was no effect of fungicide on pre-emergence mortality of *P. serotina* seedlings, although there was a site × fungicide interaction (Table 2, Fig. 4). Fungicide additions decreased pre-emergence mortality at Blm and ANF sites but had no effect on seedlings

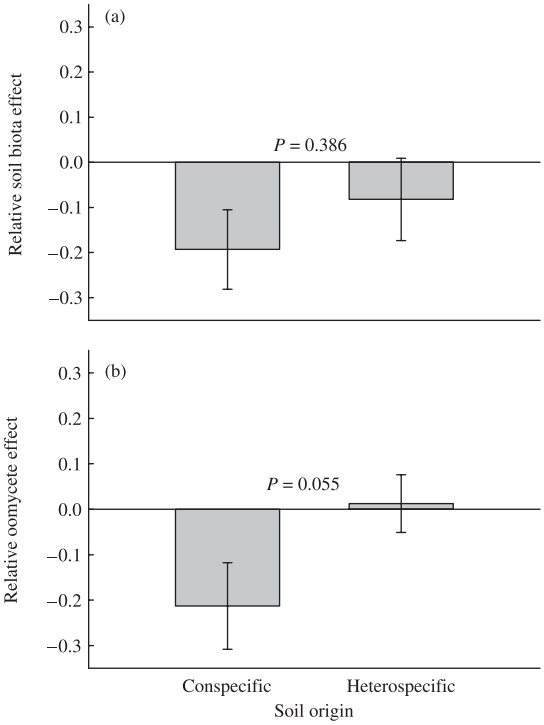

Fig. 3 Laboratory experiments testing the effect of soil origin on the relative soil biota effect (a) and relative oomycete effect (b). Bars represent means ± 1 SE. *P*-values are for one-way ANOVA results.

Table 2 Field experiment testing the effect of focal tree (near conspecific vs. near heterospecific tree), soil pathogens (no selective fungicide vs. selective fungicide), and site on average percentage pre-emergence mortality of *Prunus serotina* seedlings

Source of variation	d.f., df_{error}	*MS*, MS_{error}	*F*	*P*
Tree	1, 2.21	1.5, 244.7	0.006	0.945
Fungicide	1, 2.06	1113.2, 435.5	2.556	0.247
Tree × Fungicide	1, 3.91	84.4, 23.3	3.620	0.131
Site	2, 3.51	4237.3, 655.5	6.464	0.067
Site × Tree	2, 2	239.9, 16.7	14.350	0.065
Site × Fungicide	2, 2	432.4, 16.7	25.862	0.037
Site × Tree × Fungicide	2,28	16.7, 815.6	0.020	0.980

Notes: Emergence data within a population were averaged (n = 3–4 populations site^{-1}). *F*-values were calculated using Type III ss.

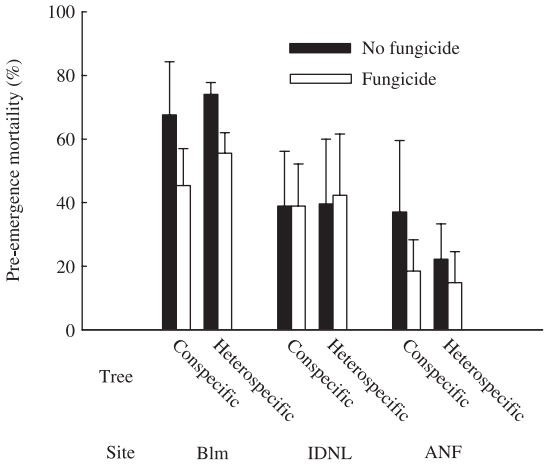

Fig. 4 Field experiment testing the effect of neighbouring trees (conspecifics vs. heterospecifics), soil pathogens (no selective fungicide vs. selective fungicide) and sites on the pre-emergence mortality of *Prunus serotina* seedlings. Sites were located at Bloomington (Blm), Indiana, Indiana Dunes National Lakeshore (IDNL), Indiana, and near the Allegheny National Forest (ANF), Pennsylvania (n = 3–4 populations site^{-1}). Bars represent means ± 1 SE. Refer to Table 2 for ANOVA results.

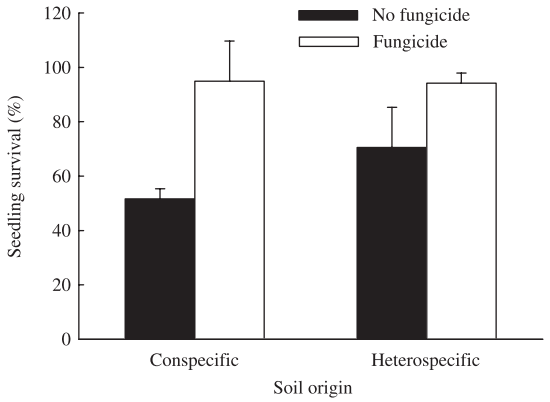

Fig. 5 Laboratory experiment testing the effect of soil origin and soil pathogens on the survival of *Prunus serotina* seedlings grown in soil collected from the Indiana Dunes National Lakeshore (n = 3 populations). Bars represent means ± 1 SE.

at IDNL. Although fungicide had no effect in the field at IDNL it increased survival in IDNL soils in the laboratory ($F_{fungicide}$ = 7.83, d.f. = 1,3, P = 0.02, Fig. 5), where it was the only factor to have an effect ($F \leq 0.47$, d.f. = 1,3, $P \geq 0.52$). Additionally, there was no effect of soil sterilization or other factors on survival of seedlings in the Blm portion of the laboratory experiment ($F \leq 0.959$, d.f. = 1,3, $P \geq 0.356$), despite a negative effect of the soil biota having been observed in a previous experiment (Packer & Clay 2000) and a positive effect of fungicide being seen in the field experiment. In the field experiment, we also observed a marginally significant effect of site and site × tree on seedling survival, but there was no effect of other factors (Table 2).

Discussion

An increasing number of community-level studies have identified the importance of negative plant–soil biota interactions in structuring plant communities at individual sites (e.g. Mills & Bever 1998; Packer & Clay 2000; Klironomos 2002; Hood *et al*. 2004). However, little is known about whether such effects occur across large spatial scales, equal to a species' range. In laboratory experiments, we found that *Prunus serotina* seedlings were generally negatively affected by the soil biota associated with conspecifics from 22 populations

located throughout the eastern USA. Specifically, we observed a positive effect of fungicide applications (suggesting a negative effect of oomycete soil pathogens) on seedling survival in the laboratory experiment and on pre-emergence mortality of germinants in the field at two of three sites located in the northern USA.

We also identified a positive effect of soil sterilization treatments on seedling survival, suggesting an overall negative effect of the soil biota. Soil sterilization is known to create a flush of nutrients and have positive effects on plant growth (Troelstra *et al*. 2001). However, we diluted this effect by sterilizing the majority of the soil (≥ 71%) in individual pots. We also used selective fungicide in field and laboratory experiments to remove the effect of oomycete pathogens while controlling for nutrient flushes associated with sterilization of all organisms in the soil. The results from the fungicide experiment are in agreement with the soil sterilization experiment testing the net effects of the soil biota (Figs 1 and 2), indicating that oomycete pathogens are probably causing the observed mortality in both the sterilization and fungicide experiments.

Previous studies have indicated that the negative effect of the soil biota on plants is host-specific (e.g. Packer & Clay 2000; Klironomos 2002; Hood *et al*. 2004). Generally, these studies were conducted at smaller spatial scales, and, typically, at one study site, albeit with greater within-site replication, and thus greater probability of observing a significant treatment × origin interaction. In contrast, in our study, only the relative oomycete effect differed between soils collected near conspecific and heterospecific trees. The small sample size used in our experiments (n = 2–3 trees population^{-1}) and the use of different tree species as heterospecifics within and between populations contributed to the variability within populations (e.g. Figure 2) and may have obscured interactive effects. Therefore, our experimental design may have been a conservative test of host-specificity and more detailed studies within sites are needed to explore this issue further. Confirming host-specific distance- and/or density-dependent mortality is important, because this interaction helps to maintain plant community diversity (Janzen 1970; Connell 1971; Packer & Clay 2000; Hille Ris Lambers *et al*. 2002).

Despite the overall positive effect of soil sterilization and fungicide treatments on seedling survival, some individual populations were negatively affected by soil sterilization and fungicide treatments. Inspection of Fig. 2 reveals one population each from three sites (Bloomington, Mammoth Cave National Park and Torreya State Park) that had positive relative oomycete effects (negative effect of fungicide) and positive relative soil biota effects (negative effect of soil sterilization). Soil sterilization and fungicide treatments may have negatively affected seedling survival in these populations by reducing/neutralizing soil microbes that functioned as strong mutualists (e.g. arbuscular mycorrhizal fungi), because pathogens resistant to the fungicide were present (Schwinn & Staub 1987) or because the fungicide was either broken down or leached from the soil before affecting the pathogens (Schwinn & Staub 1987).

In a recent study, Hood *et al*. (2004) tested the effects of both a similar fungicide (active ingredient metalaxyl) and a second fungicide on seedling mortality of *Milicia regia*, a tropical tree species, and observed a more negative effect of oomycetes associated with conspecific trees relative to heterospecific trees. In contrast to previous reports on the selectivity of metalaxyl fungicides (Schwinn & Staub 1987; Paul *et al*. 1989), Hood *et al*. (2004) found a negative effect of the metalaxyl-based fungicide on biomass of seedlings grown in non-sterilized soils that was correlated with reductions in colonization by arbuscular mycorrhizal fungi (AMF). We did not quantify AMF, but they are known to colonize the roots of *P. serotina* (Brundrett *et al*. 1990). Hood *et al*. (2004) suggested that the negative effects of soil biota on seedling survival are driven primarily by soil pathogens and not by AMF and our results are consistent with this. However, variation in seedling growth (not tested) could be confounded by this non-target effect of the fungicide on AMF. Multiple approaches to determine the individual effects of different species and functional groups of the soil biota are needed to identify the organisms and interactions structuring plant communities.

Consistent with our laboratory experiments, fungicide applications reduced pre-emergence mortality of seedlings at two of three sites in the field. The site where mortality was not affected (IDNL) has the sandiest soil of the northern sites used in this study (K.O. Reinhart, personal observation). This may be an important distinction because pathogens such as *Pythium* spp. are known to proliferate better in moist soils (Hendrix & Campbell 1973) and *P. serotina* is a successful invader on well-drained sandy soils in Europe (Reinhart *et al*. 2003). Nevertheless, the positive effect of fungicide on survival in IDNL soils in the laboratory (Fig. 5) indicates that these soils have the potential for having pathogenic effects in the field. Disease symptoms are, however, likely to depend upon environmental conditions (e.g. wet weather) that fluctuate within and between years (Hendrix & Campbell 1973; Martin & Loper 1999).

Overall, we found that sterilization of soil increased seedling survival by 33% when soil was collected from multiple *P. serotina* populations throughout the eastern USA with replicated soil communities from multiple trees. Although this was much less than the 136% reported by Packer & Clay (2000), only a coarse comparison of these studies is possible because of methodological differences (e.g. season and year that soil was collected, time stored). For example, the negative effects of the soil biota that we report for the laboratory experiment may be a conservative estimate, because we collected the soil during a period of general dormancy (winter). Storage for several months before use may also have reduced the virulent effect of the soil community (Beckstead & Parker 2003) and the strength

of plant-soil feedback may vary throughout the year (Van der Stoel *et al*. 2002). Our estimates are based on the effect of the soil community associated with individual trees rather than on metacommunities (i.e. soil communities from multiple trees bulked into one composite sample). Our experimental design, however, included considerable natural variation of soil communities as soil pathogen incidence and/or impact is likely to vary within a forest (Packer & Clay 2002).

Negative plant–soil biota interactions may affect a large percentage of tree species. For example, O'Hanlon-Manners & Kotanen (2004) found that *Betula papyrifer*, which was thought to be shade intolerant, was actually excluded from understorey habitats by the negative effects of fungal pathogens rather than by shading. The same may be true of *Prunus serotina* and other species that are often referred to as shade intolerant (Hough & Forbes 1943; Baker 1949; Auclair & Cottam 1971). The temporal and spatial dynamics of *Pythium* populations and onset of disease symptoms (i.e. damping-off) also require further investigation.

Negative plant–soil biota interactions have been shown to affect numerous types of plant communities (e.g. Van der Putten *et al*. 1993; Bever 1994; Packer & Clay 2000; Bever 2002; Klironomos 2002) but, to date, no large-scale geographical comparisons on feedbacks between plants and soil biota have been performed. The overall consistency of our laboratory and field experiments strongly suggests that oomycete soil pathogens have a negative affect on the survival of *P. serotina* seedlings throughout its native range in the eastern USA. Additional research in the native and non-native ranges is necessary to determine whether variation in plant–soil biota interactions may explain large-scale patterns of abundance, dominance, range limits and invasiveness of this and other species.

Acknowledgements

We thank two anonymous reviewers for their helpful comments. We would like to thank Kane Hardwood, a division of Collins Pine Co., for granting permission to conduct a portion of the field experiment on their property in Pennsylvania. We thank Syngenta for donating the Subdue GR. This research resulted from a post-doctoral fellowship from the USDA NRI (2003–02047) and NSF DEB-0090056.

References

Auclair, A.N. & Cottam, G. (1971) Dynamics of black cherry (*Prunus serotina* Erhr.) in southern Wisconsin oak forests. *Ecological Monographs*, **41**, 153–177.

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

Baker, F.S. (1949) A revised tolerance table. *Journal of Forestry*, **47**, 179–181.

Beckstead, J. & Parker, I.M. (2003) Invasiveness of *Ammophila arenaria*: release from soil-borne pathogens? *Ecology*, **84**, 2824–2831.

Bever, J.D. (1994) Feedback between plants and their soil communities in an old field community. *Ecology*, **75**, 1965–1977.

Bever, J.D. (2002) Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings of the Royal Society of London*, **269**, 2595–2601.

Bever, J.D. (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. *New Phytologist*, **157**, 465–473.

Brown, J.H. (1995) *Macroecology*. University of Chicago Press, Chicago, Illinois.

Brundrett, M., Murase, G. & Kendrick, B. (1990) Comparative anatomy of roots and mycorrhizae of common Ontario trees. *Canadian Journal of Botany*, **68**, 551–578.

Callaway, R.M., Thelen, G., Rodriguez, A. & Holben, W.E. (2004) Soil biota and exotic plant invasion. *Nature*, **427**, 731–733.

Connell, J.H. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forests. *Dynamics in Populations* (eds P.J. den Boer & G.R. Gradwell), pp. 298–312. Center for Agricultural Publishing and Documentation, Wageningen.

Hartnett, D.C. & Wilson, G.T. (1999) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology*, **80**, 1187–1195.

Hendrix, F.F. Jr & Campbell, W.D. (1973) *Pythium*s as plant pathogens. *Annual Review of Phytopathology*, **11**, 77–98.

Hille Ris Lambers, J., Clark, J.S. & Beckage, B. (2002) Density-dependent mortality and the latitudinal gradient in species diversity. *Nature*, **417**, 732–734.

Hood, L.A., Swaine, M.D. & Mason, P.A. (2004) The influence of spatial patterns of damping-off disease and arbuscular mycorrhizal colonization on tree seedling establishment in Ghanaian tropical forest soil. *Journal of Ecology*, **92**, 816–823.

Hough, A.F. & Forbes, R.D. (1943) The ecology and silvics of forests in the high plateaus of Pennsylvania. *Ecological Monographs*, **13**, 299–320.

Janzen, D.H. (1970) Herbivores and the number of tree species in tropical forests. *American Naturalist*, **104**, 501–528.

Klironomos, J.N. (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, **417**, 67–70.

Knevel, I.C., Lans, T., Menting, F.B.J., Hertling, U.M. & Van der Putten, W.H. (2004) Release from native root herbivores and biotic resistance by soil pathogens in a new habitat both affect the alien *Ammophila arenaria*, South Africa. *Oecologia*, **141**, 502–510.

Little, E.L. Jr (1977) *Atlas of United States trees*. Miscellaneous Publication 1342. US Department of Agriculture, Washington, DC.

Markham, J.H. & Chanway, C.P. (1996) Measuring plant neighbour effects. *Functional Ecology*, **10**, 548–549.

Marquis, D.A. (1990) *Prunus serotina* Ehrh. black cherry. *Silvics of North America* (eds R.M. Burns & B.H. Honkala), pp. 594–604. US Department of Agriculture, Forest Service, Washington, DC.

Martin, F.N. & Loper, J.E. (1999) Soilborne plant diseases caused by *Pythium* spp. Ecology, epidemiology, and prospects for biological control. *Critical Reviews in Plant Sciences*, **18**, 111–181.

Mills, K.E. & Bever, J.D. (1998) Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. *Ecology*, **79**, 1595–1601.

O'Hanlon-Manners, D.L. & Kotanen, P.M. (2004) Evidence that fungal pathogens inhibit recruitment of a shade-intolerant tree, white birch (*Betula papyrifera*), in understory habitats. *Oecologia*, **140**, 650–653.

Olff, H., Hoorens, B., de Goede, R.G.M., Van der Putten, W.H. & Gleichman, J.M. (2000) Small-scale shifting mosaics of

two dominant grassland species: the possible role of soil-borne pathogens. *Oecologia*, **124**, 45–54.

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

Packer, A. & Clay, K. (2002) Soil pathogens and *Prunus serotina* seedlings and sapling growth near conspecific trees. *Ecology*, **84**, 108–119.

Packer, A. & Clay, K. (2004) Development of negative feedback during successive growth cycles of black cherry. *Proceedings of the Royal Society of London*, **271**, 317–324.

Paul, N.D., Ayres, P.G. & Wyness, L.E. (1989) On the use of fungicides for experimentation in natural vegetation. *Functional Ecology*, **3**, 759–769.

Reinhart, K.O. & Callaway, R.M. (2004) Soil biota facilitate exotic *Acer* invasion in Europe and North America. *Ecological Applications*, **14**, 1737–1745.

Reinhart, K.O., Packer, A., Van der Putten, W.H. & Clay, K. (2003) Plant–soil biota interactions and spatial distribution of black cherry in its native and invasive ranges. *Ecology Letters*, **6**, 1046–1050.

Reynolds, H.L., Packer, A., Bever, J.D. & Clay, K. (2003) Plant–microbe–soil interactions as drivers of plant community structure and dynamics. *Ecology*, **84**, 2281–2291.

Schwinn, F.J. & Staub, T. (1987) Phenylamides and other fungicides against oomycetes. *Modern Selective Fungicides-Properties, Application, Mechanisms of Action* (ed. H. Lyr), pp. 260–273. Longman, London.

Troelstra, S.R., Wagenaar, R., Smant, W. & Peters, B.A.M. (2001) Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. *New Phytologist*, **150**, 697–706.

Van der Putten, W.H., Van Dijk, C. & Peters, A.M. (1993) Host-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature*, **362**, 53–56.

Van der Stoel, C.D., Van der Putten, W.H. & Duyts, H. (2002) Development of a negative plant-soil feedback in the expansion zone of the clonal grass *Ammophila arenaria* following root formation and nematode colonization. *Journal of Ecology*, **90**, 978–988.

Wardle, D.A. (2002) *Communities and Ecosystems: Linking the Aboveground and Belowground Components*. University Press, Princeton, New Jersey.

Zhang, B.Q. & Yang, X.B. (2000) Pathogenicity of *Pythium* populations from corn-soybean rotation fields. *Plant Disease*, **84**, 94–99.

Received 31 December 2004
revision accepted 7 April 2005
Handling Editor: Jeremy Burdon