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Entomopathogen biodiversity increases host mortality

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ABSTRACT

In biological control communities, greater predator species richness often strengthens pest suppression. The impacts and importance of species richness in insect-killing pathogen (entomopathogen) communities, however, has received less attention. Here, we manipulated species richness within a community of three soil-dwelling entomopathogenic nematodes (*Heterorhabditis megidis*, *Steinernema carpocapsae*, *Steinernema feltiae*) and one fungus (*Beauveria bassiana*), and measured resulting effects on mortality of Colorado potato beetle (*Leptinotarsa decemlineata*) and wax moth (*Galleria mellonella*) hosts. When potato beetles were the focal host, increasing pathogen species richness led to a linear increase in host mortality. This diversity effect appeared to result primarily from the pairing of nematodes and fungus within diverse communities; these pairings always produced host mortality that exceeded predictions based on the impact of nematodes or fungus alone. We then conducted an experiment using wax moth as the focal host, because this allowed greater replication, and included two different soil types to see if changing the foraging environment altered niche differences (and thus complementarity) among pathogen species. We again found an increase in host mortality at higher pathogen richness levels due to the apparent nematode–fungus synergism, and this effect was detected across both soil types. Our findings suggest that the positive effects of species richness commonly observed among predator species may extend to pathogen communities as well, such that conserving pathogen biodiversity may carry additional benefits for biological control.

1. Introduction

It is well established that increasing biodiversity improves many ecosystem processes of ecological and socio-economic importance, such as biological control, resistance to invasion, and resilience to disturbance (e.g., Cardinale et al., 2006; Chapin et al., 1997; Hooper et al., 2005; Loreau et al., 2001). Experimental manipulations across a variety of systems have shown that increases in the number of consumer species (richness) generally increases total resource use across the entire community (Cardinale et al., 2006; Hooper et al., 2005). The same can hold true when predators are the consumers, and agricultural pests the resource, with greater predator species richness often strengthening pest suppression and increasing plant yields (e.g., Cardinale et al., 2003; Snyder et al., 2006). For predators, greater species richness improves pest suppression through complementarity, where different enemy species attack different subsets of the prey population (Finke and Snyder, 2008, 2010; Straub et al., 2008), or facilitation, where the presence of one predator increases prey capture by a second predator species (Sih et al., 1998). When complementarity and/or facilitation is strong, the combined effects of multiple predators on their prey can exceed what any single predator species can achieve on its own, at any density (Northfield et al., 2010).

Despite their great biodiversity (Adams et al., 2006; Grewal et al., 2005) and obvious importance as herbivore natural enemies (Hawkins et al., 1997), relatively few biodiversity experiments have included pathogens (Crowder et al., 2010). In several cases, entomopathogens have been shown to exert impacts on herbivores that complement those of predators. For example, both generalist predators and specialist pathogens are important for control of some forest-insect pests, such that pest densities are determined by both host–pathogen cycles and stabilizing effects of predators (Dwyer et al., 2004). Likewise, Ramirez and Snyder (2009) found that increasing species richness among predators and pathogens attacking Colorado potato beetle (*Leptinotarsa decemlineata*) improved control of this pest, in part because predators and pathogens complement one another by attacking different beetle stages in different habitats. In other cases, however, pathogens can infect predators and disrupt biological control (Rosenheim, 1998). In turn, the small handful of studies examining species richness effects entirely within entomopathogen communities have produced mixed results. Barbercheck and Kaya (1991) found that

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prior exposure to the fungal pathogen *Beauveria bassiana* rendered caterpillars (*Spodoptera exigua*) more susceptible to later infection by the entomopathogenic nematode *Heterorhabditis bacteriophora* (see also Ansari et al., 2006). In contrast, Shapiro-Ilan et al. (2004) found general antagonism when pairs of nematode and fungal pathogens attacked weevil (*Curculio caryae*) larvae, such that most pairings were less effective than single highly effective species. This antagonism may have resulted from negative interactions between microbes or their toxins prior to or during the infection process. In several other studies, pathogen species pairs produced simple additive effects, such that the combined impact of any two species could be predicted from the monoculture performance of each pathogen species when alone (Ansari et al., 2006; Neumann and Shields, 2008). Thus, across these many studies, there is no clear pattern for pest control to strengthen, weaken, or remain unchanged when more than one pathogen species is present together. What has been missing, however, is a systematic comparison of simple versus diverse pathogen communities, across many pathogen richness levels and species compositions, to search for any general pathogen richness effects that span the outcomes of particular species pairings (Ramirez and Snyder, 2009).

Here, we examine the relationship between species richness among a community of soil-dwelling entomopathogens, and their resulting impacts on Colorado potato beetle and wax moth (*Galleria mellonella*) hosts. We considered three entomopathogenic nematode species, *Heterorhabditis megidis*, *Steinernema carpocapsae*, and *Steinernema feltiae*, and one fungal entomopathogen, *Beauveria bassiana*; these taxa co-occur in potato (*Solanum tuberosum*) and other crops in the northwestern USA, where they act together to suppress Colorado potato beetles and other insect pests with subterranean stages (Liu and Berry, 1995; Ramirez et al., 2009). These pathogen species differ from one another in many ecologically important ways, which might lead them to complement one another when attacking hosts (Lewis et al., 2006). For example, *Steinernema carpocapsae* deploys a "sit and wait" ambusher attack style, waiting to attack hosts that pass nearby, whereas *Heterorhabditis megidis* is a "cruiser" that actively moves through the soil hunting for hosts (Kruitbos et al., 2010). Likewise, species in the *Heterorhabditis* genus are often detected deeper in the soil profile than *S. carpocapsae* in the field (Millar and Barbercheck, 2001). These differences might lead to different entomopathogen species attacking hosts in different times or places, encouraging complementarity.

2. Materials and methods

2.1. Organisms used in our experiments and experimental arenas

Nematodes were provided by Becker Underwood (Ames, IA, USA) in a gel formulation that was dissolved in water. *Beauveria bassiana* was provided by Bioworks, Inc. (Victor, NY, USA) as the product Mycotrol-O, which contains free-living spores in aqueous solution. Pipettors were used to apply aqueous solutions of nematodes and fungi to each experimental replicate. Potato beetles were reared to the 4th instar in a greenhouse on potato plants for use in experiment one (25 °C, 50% R.H., ambient light), and field collected from potato plantings as 4th instar larvae for use in experiment two. Wax moths (*Galleria mellonella*) were purchased as larval waxworms (Nature's Way, Ross, OH, USA).

Each experimental replicate consisted of a 1L plastic cup, filled with 400 g of autoclaved soil and covered with a clear plastic lid after pathogens and hosts were added (described below). This soil was collected from the Washington State University, Tukey Orchard in Pullman, WA (Table 1), unless stated otherwise. Based on typical soil moisture levels during the field season (Ramirez, 2008), we standardized soil moisture at 10% in each experimental replicate by adding an appropriate amount of distilled water at the same time that pathogens were inoculated into the soil. Microcosms were housed in the laboratory at room temperature with ambient light.

Table 1
Characteristics of soil collected from Pullman, WA (Pullman) versus soil collected from Othello, WA (Othello). Pullman soil was used in all experiments, whereas Othello soil was used only in the third experiment where pathogen diversity effects were compared across the two soil types. These data are the mean of two representative soil sub-samples of 400 g, removed from the soil used in our experiments.

	Pullman	Othello
NO_3–N (ppm)	130.5	310.5
NH_4–N (ppm)	12.25	25.75
P Bicarb (ppm)	79	50
K Bicarb (ppm)	384	313
SO_4–S (ppm)	21	19.5
B (ppm)	1.985	0.255
OM (%)	4.22	1.445
pH	6.85	6.3
SS (mmho/cm)	0.73	0.76
Zn (ppm)	4.35	3.3
Mn (ppm)	89	14
Cu (ppm)	1.65	1.6
Fe (ppm)	26	26
Ca (meq/100 g)	13.3	6.65
Mg (meq/100 g)	3.7	1.95
Na (meq/100 g)	0.125	0.16
% Sand	25	33
% Silt	68.4	61.9
% Clay	6.6	5.1

2.2. Pathogen richness effects on potato beetle mortality

We conducted two experiments wherein we examined whether entomopathogen species richness impacted mortality of potato beetle larvae. The first experiment included a relatively broad gradient of species richness (1 versus 4 pathogen species), but included just one diverse species composition (all four pathogen species together). The second experiment spanned a smaller range of richness levels (1, 2, or 3 pathogen species), but included all possible combinations of pathogen species at each level of species richness.

In the first experiment including potato beetles as hosts, we constructed two species-richness treatments: single species – each of the four entomopathogen species present alone; and diverse – a polyculture including all four entomopathogen species. Pathogen species richness was manipulated within a substitutive design, such that total pathogen densities were the same across the single species and diverse treatments. To achieve this, pathogens were applied at densities of 1000 nematodes or 4×10^7 fungal spores, with one-fourth these amounts of each species in the diverse, 4-species treatment. These densities approximately reproduce the level of insect infection seen in local potato fields (Ramirez and Snyder, 2009). After pathogens were applied, we added 10 late-4th-instar potato beetle larvae to each microcosm, along with potato foliage so that the larvae could feed and bury in the soil naturally. We allowed 3 weeks for beetles to complete pupation, then counted the number of adults that had emerged (the soil in each microcosm was searched to ensure that no living beetle pupae remained). Each diversity level was replicated 8 times, with two replicates of each of the four single-species compositions (that is, each species was present by itself in two different replicate arenas), and eight replicates of the fully diverse community. In addition, there were four control replicates with no pathogens, for a total of 20 experimental units within each temporal block. The experiment was conducted

twice, at different times (the first block was conducted in December 2009, and the second block in March 2010), for a total of 40 experimental units (2 blocks × 20 replicates per block = 40).

In the second experiment using potato beetle larvae as hosts, we established three levels of pathogen species richness from a pool of species including *S. carpocapsae*, *S. feltiae*, and *B. bassiana* (*Heterorhabditis megidis* was excluded in this experiment because it killed the fewest potato beetles in experiment one): (1) each of three pathogen species present alone in separate microcosms; (2) each possible paired combination of the three pathogen species established in separate microcosms; and (3) all three pathogen species established together. A substitutive design was again used, with pathogen application rates as described above except that inoculum of each species was divided by 2 in the 2-species treatment, and by 3 in the 3-species treatment, to again maintain constant total pathogen densities across diversity levels. Potato beetle larvae were added to arenas and then later scored for mortality at the adult stage as described above. We conducted 10 replicates of each species in monoculture (3 species × 10 replicate monocultures = 30), 10 replicates of each possible combination of two species from the pool of three (three unique combinations of 2 species × 10 replicates = 30), and 10 replicates of the diverse community with all three species. We also included five control replicates with no pathogens, for a total of 75 experimental units (30 in monoculture + 30 with 2 species + 10 with 3 species + 5 controls = 75). This experiment was conducted in September 2010.

2.3. Pathogen richness effects on waxworm mortality in two soil types

Our third experiment had two objectives. First, we wished to ascertain whether pathogen diversity effects were similar when waxworms, rather than potato beetles, served as focal hosts. Waxworms can be ordered in large quantities, cheaply, from commercial suppliers. If waxworms serve as a suitable stand-in for potato beetles, this makes it relatively easy to conduct large, complex experiments that require many host insects. Second, we wished to examine whether soil type would influence the strength or nature of pathogen diversity effects. This could occur because different environments might encourage or suppress niche differences among pathogen species, weakening, or strengthening complementarity (e.g., Griffin et al., 2009). On the other hand, if diversity effects are consistently strong across environments, this indicates relatively stable niche differences among pathogen species (at least, across the two soil environments that we considered).

Here, arenas received either the Pullman soil described above, or soil from the WSU Experimental Research Station in Othello, WA. These two soils differed in a number of characteristics (Table 1). This experiment included the four pathogen species described above, in all possible combinations, at each of four levels of species richness (1, 2, 3, or 4 species). We included four replicates of each pathogen species in monoculture (4 species × 4 replicates per species = 16 total arenas); three replicates of each of the six possible pairs of pathogen species drawn from the pool of four species (6 possible pairs of species × 3 replicates per pair = 18 total arenas); three replicates of each of the possible draws of three pathogen species from the pool of four species (4 possible pathogen species trios × 3 replicates of each trio = 12 total arenas); and four replicates of all four pathogen species present together. The design also included four no-pathogen controls. This led to a total of 54 replicates within each soil type, and a total of 108 replicates across the two soil types. The experiment was then repeated as a second temporal block, for a total of 216 experimental replicates across the entire experiment (108 replicates per temporal block × 2 temporal blocks = 216). These blocks were conducted in September and October 2009.

Pathogen species richness was again manipulated within a substitutive design. Each monoculture received either 120 nematodes or 4.8×10^6 fungal spores, with one-half, one-third, or one-fourth these densities of each species added to the 2, 3, and 4 species treatments, respectively. These pathogen densities equalize waxworm mortality between nematode and fungus species (Ramirez et al., 2009). After adding pathogens, we added 20 waxworms to each replicate, stored the microcosms in the dark at room temperature for one week, and then recollected waxworms and assessed mortality.

2.4. Data analysis

Mortality in treatments with pathogens was corrected for control mortality (Abbott, 1925). In each experiment, within each block or each block and soil type in the third experiment, we calculated the mean survival across all control replicates. Then, for each replicate, we calculated adjusted mortality as *adjusted mortality* $= 1 - (1 - X)/(1 - Y)$, where X = proportion survival in treated replicate and Y = mean proportion survival of controls. For the first experiment using potato beetles as hosts, we used ANOVA to evaluate whether pathogen richness (single species or diverse), block, and the interaction term, affected mortality of potato beetles. For the second experiment using potato beetles as hosts, we used linear regression to test the impact of the number of pathogen species on host mortality. In the third experiment, where waxworms were the hosts and two soil types were examined, we used multiple regression to evaluate the impacts of the number of pathogen species, soil type (indicator variable), block (indicator variable), and all two-way interactions, on host mortality. For each analysis, we used a conservative data-analysis approach, whereby all replicates within a given level of richness were pooled, which allowed us to examine the role of diversity *per se* and limit confounding species-identity effects (Snyder et al., 2006). Therefore, each richness level included all possible species compositions, and composition itself was not included in the analysis. Adjusted mortality was arcsine-square root transformed prior to each analysis to meet assumptions of homogeneity of variance and normality.

Initial examination of the data suggested that diversity effects were being driven by pairings of nematodes with *B. bassiana*. These pairings appeared to consistently kill more beetles than would be expected based on what either the nematode or fungus could accomplish when present alone. To evaluate the potential for synergism between nematodes and fungi, we tested for emergent biodiversity effects (overyielding) in the host mortality data, comparing multi-species communities that brought together two or three nematode species to those including both at least one nematode species and *B. bassiana*. We calculated D_T, a metric of polyculture performance relative to the average of its constituent species in monoculture (Petchey, 2003). D_T values greater than zero indicate that a diverse community killed more insects than expected based on single-species performance, whereas values less than zero indicate poorer performance than expected. For the jth polyculture, the D_T value was calculated as $D_{Tj} = (O_j - E_j)/E_j$, where O_j = observed mortality in polyculture j and E_j = expected mortality in polyculture j; E_j = average mortality in monoculture across all species contained in polyculture j. We used Wilcoxon signed rank tests to determine if the median D_T value differed significantly from zero (as D_T values were highly non-normal). Values significantly different than zero indicate evidence of emergent diversity effects (that is, the ability of polycultures to kill hosts was significantly greater or less than expected based on what each pathogen species killed when alone). We compared D_T values for single species and diverse communities using Kruskal–Wallis χ^2 tests.

Overyielding can result from complementarity or facilitation among species, or can result from species-identity effects, whereby

diverse communities simply contain the most effective species by chance alone (Loreau et al., 2001). To examine the potential for species-identity effects, we conducted additional analyses to determine if particular single-species treatments in each experiment caused mortality similar to the most diverse communities. Thus, these analyses differed from those described previously by analyzing mortality in each single-species monoculture rather than pooling across species. For each experiment, we used ANOVA followed by LSD *post hoc* tests to evaluate whether each single-species treatment caused mortality similar to the most diverse community (i.e., the one with the most species). In the first experiment with potato beetles as the focal hosts, block and the interaction between block and treatment were included along with treatment as explanatory variables; in the waxworm experiment block, soil type, and all two-way interactions were included along with treatment as explanatory variables. Statistical analyses were conducted in JMP (SAS Institute, 2007).

3. Results

3.1. Pathogen richness effects on potato beetle hosts

Our first experiment using potato beetles as hosts showed that significantly fewer beetles were killed across the species monocultures compared with the diverse pathogen community with all four species (Fig. 1A; $F_{1,28} = 23.9$, $P < 0.0001$). This diversity effect was consistent across the two temporal blocks (Interaction term: $F_{1,28} = 0.82$, $P = 0.37$), although overall mortality was higher in Block 1 than Block 2 (Fig. 1A; $F_{1,28} = 14.2$, $P = 0.001$). Similarly, in the second experiment with potato beetles as hosts, increasing pathogen species richness resulted in a nearly perfectly linear increase in potato beetle mortality (Fig. 1B; $F_{1,68}$=35.0, $P < 0.0001$).

There was evidence for significant overyielding in the first experiment with potato beetle hosts, as D_T values were significantly greater than 0 (Mean = 0.52, $SE = 0.16$, $SR^+ = 53.0$, $P = 0.0038$). Similarly, in the second experiment with potato beetle hosts, all diverse compositions killed significantly more beetles than would be expected based on the performance of species in monoculture, indicating emergent diversity effects (Fig. 2; two-nematode species: $SR^+ = 20.5$, $P = 0.037$; one nematode and fungus: $SR^+ = 0.92$, $P = 0.0002$; three species: $SR^+ = 27.5$, $P = 0.002$). These emergent diversity effects were stronger when *B. bassiana* was present in the community than when it was absent, with both the nematode–*B. bassiana* pairs ($\chi^2 = 4.50$, $df = 1$, $P = 0.034$) and the three-species communities ($\chi^2 = 10.2$, $df = 1$, $P = 0.0014$) performing better than pairings that brought together the two-nematode species (Fig. 2).

The overyielding seen in diverse communities containing *B. bassiana* was not because the fungus was invariably a particularly lethal pathogen, as *B. bassiana* produced significantly lower mortality in monoculture than was exerted by the diverse communities (Fig. 3A, B). In the first experiment with potato beetles, *S. carpocapsae* in monoculture produced mortality that was not significantly different than the diverse community, although each other species produced mortality lower than the diverse community (Fig. 3A). No single species produced mortality as high as the diverse community in the second experiment with potato beetles (Fig. 3B), suggesting that identity effects associated with any particular species were not pervasive when potato beetle was the focal host.

3.2. Pathogen richness effects on waxworms and influence of soil type

Increasing pathogen species richness increased waxworm mortality (Fig. 4; Table 2). Although mortality varied between blocks, there was no significant interaction between pathogen richness and block (Fig. 4; Table 2), suggesting that richness effects were

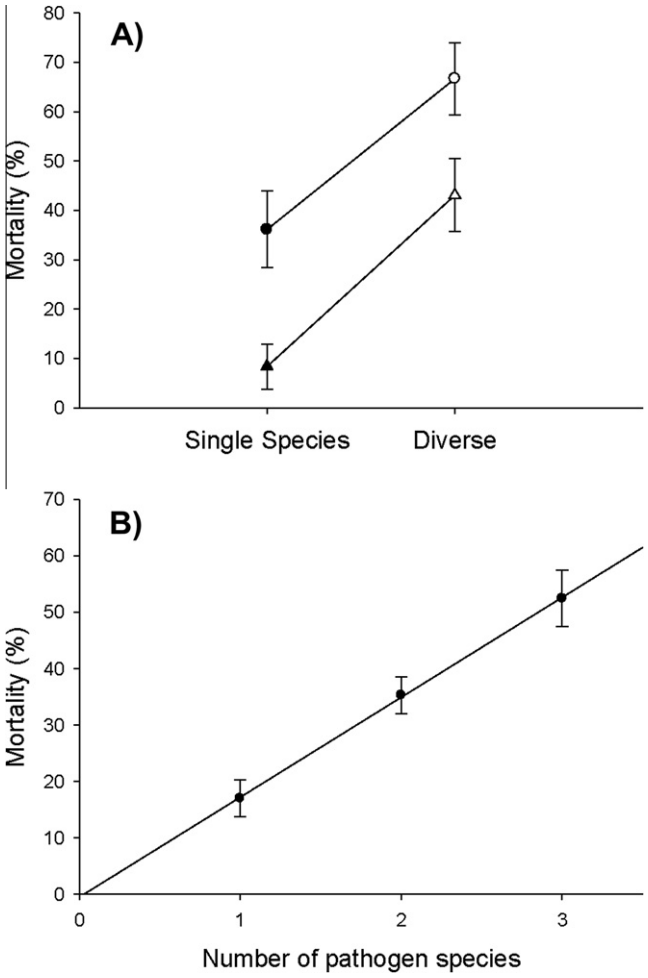

Fig. 1. (A) Effects of single pathogen species or four pathogen species together (diverse) on *Leptinotarsa decemlineata* mortality in temporal blocks 1 (circles) and 2 (triangles) of the first experiment. (B) Effects of the number of pathogen species on *Leptinotarsa decemlineata* in the second experiment.

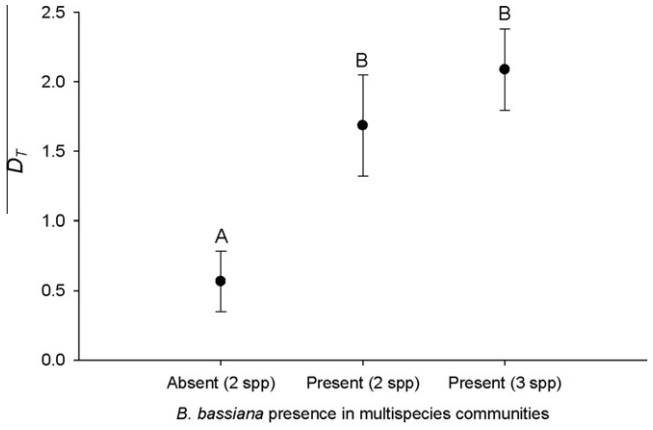

Fig. 2. D_T (overyeilding) values (Mean ± *SE*) in the second experiment with potato beetle hosts for multi-species communities including two species of entomopathogenic nematode but no fungal pathogen (Absent [2 spp.]), one species of entomopathogenic nematode and the fungal pathogen *Beauveria bassiana* (Present [2 spp.]), or *B. bassiana* along with two nematode species (Present [3 spp.]). In all cases, D_T values were significantly greater than zero ($P < 0.05$), indicating emergent biodiversity effects. Emergent biodiversity effects were significantly stronger when *B. bassiana* was included in the community than when this species was not paired with at least one entomopathogenic nematode species ($\chi^2 = 10.5$, $df = 2$, $P = 0.0053$) (as indicated by letters above error bars).

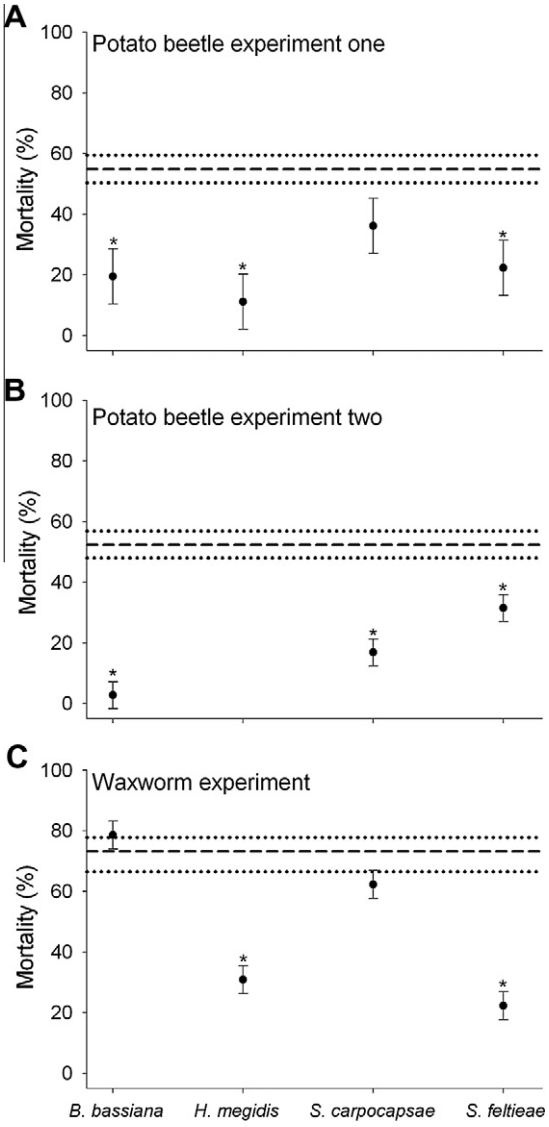

Fig. 3. Potato beetle *Leptinotarsa decemlineata* mortality in the (A) first and (B) second experiment, and (C) waxworm *Galleria mellonella* mortality due to each pathogen species in monoculture (LS Means ± *SE*) compared with the most-species rich community (four species in experiment 1 with potato beetles; three species in experiment 2 with potato beetles; four species in waxworm experiment). In each panel, the mean of the diverse community is shown with dashed line, where dotted lines indicate ± 1 SE. Asterisks indicate that the single-species monoculture produced significantly lower mortality than the diverse community (ANOVA with LSD *post hoc* tests, $\alpha = 0.05$).

robust. Soil type exerted no main or interactive effects on waxworm mortality (Fig. 4; Table 2), indicating that pathogen diversity effects were consistently strong and positive regardless of foraging environment. Positive emergent diversity effects were always detected for multi-species communities including *B. bassiana*, but no significant emergent diversity effects were detected for nematode-only communities (Table 3). Contrary to the experiments with potato beetle as the focal host, *B. bassiana* and *S. carpocapsae* in monoculture caused mortality similar to the diverse communities (Fig. 3C). Thus, we could not exclude the possibility that overyielding in nematode–fungus pairings with waxworms as the focal host may have been due to the inclusion of the single most-impactful species, *B. bassiana*, within diverse communities, rather than reflecting complementarity or facilitation among pathogen species more generally.

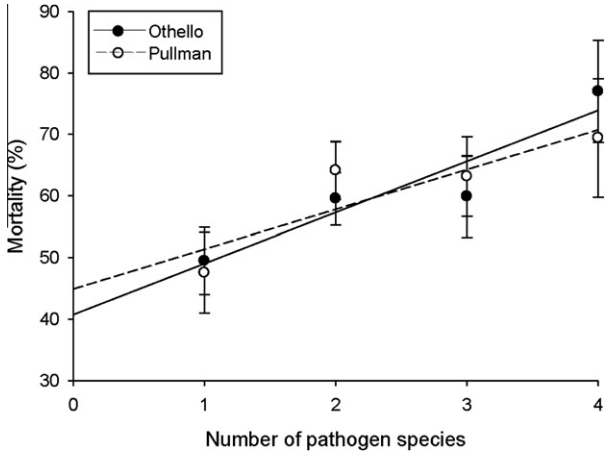

Fig. 4. Waxworm (*Galleria mellonella*) mortality (means ± SE), along with the best-fit regression lines, when facing infection by four levels of pathogen species richness within soils collected from Othello, WA (Othello; black circles) and soil collected from Pullman, WA (Pullman; open circles).

Table 2
Multiple regression analysis of pathogen richness effects on waxworm mortality across two soil types and two temporal blocks.

Effect	Estimate	*SE*	t_{193}	*P*
Pathogen richness	0.091	0.026	3.55	0.0005
Soil type	0.0045	0.048	0.09	0.92
Block	−0.38	0.048	−7.98	<0.0001
Richness × soil	0.012	0.051	0.24	0.81
Richness × block	−0.090	0.051	−1.76	0.080
Soil × block	−0.15	0.096	−1.58	0.12

Table 3
Emergent biodiversity effects in waxworm richness experiment.

Number of species	*B. bassiana*	Median D_T	SR^+ test statistic	*P*
Two	Present	0.4	317.0	<0.0001
Three	Present	0.27	165.5	0.0074
Four	Present	0.38	65.0	0.0002
Two	Absent	0.052	113.5	0.074
Three	Absent	0.12	18.0	0.17

4. Discussion

There is growing evidence that greater species richness among natural enemies can improve pest suppression (Finke and Snyder, 2010; Straub et al., 2008). These studies generally have focused on richness effects among predators (Griffin et al., 2008; Griffiths et al., 2008; Sokol-Hessner and Schmitz, 2002; Straub and Snyder, 2006; Wilby et al., 2005), parasitoids (Finke and Snyder, 2008; Tylianakis et al., 2007), or mixed groups of these two natural enemy classes (Cardinale et al., 2003; Snyder et al., 2006). It is perhaps surprising that so few enemy-biodiversity experiments have included insect-killing pathogens, despite the key role that these natural enemies play in suppressing herbivore populations (Hawkins et al., 1997). We examined the relationship between species richness among a guild of entomopathogenic nematodes and fungi, important natural enemies of Colorado potato beetle in potato fields in the northwestern US (Armer et al., 2004; Berry et al., 1997; Ramirez et al., 2009), and their combined impacts on host insects. When either potato beetles or waxworms were the focal hosts, we saw a roughly linear increase in host mortality as pathogen species richness increased (Figs. 1 and 4). Interference

among predator species sometimes leads to reduced pest suppression as disruptive intraguild predators are added to diverse communities (Finke and Denno, 2004), and there is some evidence for persistent interference among pathogen species (Shapiro-Ilan et al., 2004). Among the four pathogen species that we considered, however, diversity effects were consistently positive.

While positive diversity effects were pervasive in our experiments, the specific mechanism(s) underlying this pattern remain unclear. When potato beetles were the focal host, all combinations of multiple species exhibited D_T (overyielding) values significantly greater than zero (Fig. 2). This means that all combinations of two or more pathogen species killed more potato beetles than would be expected based on the number of beetles killed by those same pathogen species when present alone. Among predators and other consumers, emergent diversity effects of this type are thought to result from some combination of two non-mutually exclusive mechanisms: complementarity, where different species use somewhat different subsets of the resource pool such that the greatest overall resource use is seen when multiple species co-occur; and facilitation, where the presence of one consumer species indirectly improves resource consumption by a second species (Hooper et al., 2005; Straub et al., 2008). Unfortunately, complementarity and facilitation produce nearly identical patterns of multi-enemy prey consumption such that the two mechanisms are very difficult to differentiate from one another (Northfield et al., 2010). There is reason to think that multiple pathogen species might both complement and facilitate one another's ability to infect hosts. Different entomopathogen species utilize different host-location strategies and occupy different soil strata (Koppenhofer et al., 1996; Neumann and Shields, 2006), both of which would tend to encourage spatio-temporal partitioning of the prey resource and thus complementarity (Wilby and Thomas, 2002). Likewise, there is some evidence that prior exposure to one natural enemy species can drain host resources allotted to immune defense, making subsequent infection by a second entomopathogen species more likely (Ramirez and Snyder, 2009; Steinhaus, 1958). Either or both of these mechanisms could be contributing to our results, and further research will be needed to precisely elucidate the factors underlying consistently positive diversity effects in our experiments.

Notable in our data was a particularly dramatic increase in host mortality when an entomopathogenic nematode species, regardless of the species of nematode, was combined with the fungal pathogen *Beauveria bassiana* (Fig. 2, Table 3). As with the diversity effect that we observed more generally across our pathogen community, this particularly strong diversity effect could result from nematode–fungus complementarity, facilitation, or both. Complementarity often grows with increasing phylogenetic distance among species, reflecting the many ecological differences that accumulate as lineages diverge (Cadotte et al., 2008). Clearly, among the species that we examined the nematodes and fungus are most distantly related and so likely to be the most ecologically dissimilar. For example, *B. bassiana* infects hosts through passive, chance contact between sedentary fungal spores and insects moving on or through the soil, whereas the nematodes have some mobility and thus ability to actively hunt for hosts (Hajek and Stleger, 1994; Lewis et al., 2006). These obvious host-location differences between the nematodes and *B. bassiana* could encourage members of the two pathogen phyla to attack different, and complementary, subsets of the host-insect population (Ramirez and Snyder, 2009; Straub and Snyder, 2008; Wilby and Thomas, 2002; Wilby et al., 2005). Additionally, experiments have shown that nematodes prefer to attack healthy hosts rather than those already infected with *B. bassiana* (Barbercheck and Kaya, 1991), and that nematodes and *B. bassiana* exhibit optimal growth at different temperatures (Barbercheck and Kaya, 1990); both factors would naturally encourage nematode–fungus complementarity. There also is evidence that nematodes and *B. bassiana* may facilitate one another's ability to infect hosts. For example, Ansari et al. (2006) proposed that a host-insect that is battling fungal infection may become stressed and sluggish, and thus potentially more susceptible to nematode infection, a supposition with some experimental support (e.g., Barbercheck and Kaya, 1991; Ansari et al., 2006). Increased mortality in diverse communities pairing nematodes and fungus could also be due to species-identity effects, given that diverse communities are more likely to include a particularly effective natural enemy (Loreau et al., 2001). With potato beetle as the focal host, diverse communities produced higher mortality than single species, suggesting that identity effects were not the sole factor driving positive biodiversity effects (Fig. 3A, B). With waxworms as the focus host, however, two pathogen species in monoculture produced mortality similar to the diverse community, suggesting that species-identity effects may contribute to the positive diversity effects seen with waxworms (Fig. 3C).

In an experiment using waxworms as the focal host, we found no evidence that the benefits of pathogen biodiversity were impacted by the soil environment (Fig. 4). In other systems, more complex environments have been shown to heighten complementarity among consumer species, by allowing species-specific differences in foraging behavior to be displayed and accentuated (Griffin et al., 2009). While the two soil types that we considered did differ in several characteristics (Table 1), it is possible that these differences either were not sufficiently large to impact host location by the pathogen species, or that niche differences among pathogen species are resilient to background soil structure across this range of soil variability.

Alternatively, the benefits of biodiversity could be impacted by the complexity of the soil environment. Griffin et al. (2009) tested for effects of heterogeneity by combining substrate types within a mesocosm; perhaps increased structural complexity via additions of residue or plant roots would impact the benefits of pathogen biodiversity. Our experiments were conducted in relatively benign and constant temperature and soil moisture conditions in the laboratory, and it is possible that the broader range of conditions experienced in the field would reveal niche differences among pathogen species not revealed in our simple laboratory microcosms (Hunt et al., 2001; Jabbour and Barbercheck, 2008; Studdert et al., 1990).

In summary, we found a remarkably pervasive increase in host mortality with increasing species richness among entomopathogen species. These biodiversity benefits spanned the particular combinations of pathogen species, the level of species richness, and soil type. Our work was conducted in laboratory microcosms, but if our results translate to field conditions this suggests that conservation or augmentation of pathogen biodiversity would benefit biological control. When predatory arthropods are the foci of conservation efforts, a wide variety of techniques have been proposed to make agricultural fields more hospitable to a diverse group of predator and parasitoid species (Landis et al., 2000). Less attention has been paid to the conservation of entomopathogens, but there is some evidence that greater biodiversity can also be encouraged among these natural enemies (Crowder et al., 2010). For example, replacing broad-spectrum synthetic chemical fumigants with bio-fumigants or other non-chemical alternatives can encourage greater abundance and diversity of entomopathogens (Martin, 2003; Matthiessen and Kirkegaard, 2006; Ramirez et al., 2009). Similarly, building soil organic matter through greater use of organic manures and cover-cropping can foster more diverse soil food webs, including greater entomopathogen activity (Duncan et al., 2007; Kaya and Koppenhöfer, 1996). Finally, reducing tillage intensity maintains the complex soil structure that allows niche differences among entomopathogens to be fully realized (Millar and Barbercheck, 2001).

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