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The composition of soil-dwelling pathogen communities mediates effects on wireworm herbivores and wheat productivity

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ABSTRACT

Greater natural enemy diversity generally increases prey mortality. Diversity can be beneficial when natural enemy species occupy distinct niches (complementarity effects) or when diverse communities contain the most impactful species by chance (identity effects). Most research assessing effects of natural enemy diversity focuses on aboveground predators, however, and few studies have assessed whether increased entomopathogen diversity in soil affects belowground herbivores. Here, we assessed effects of entomopathogen richness on herbivore suppression and plant productivity in a system consisting of entomopathogens, wireworm herbivores, and wheat plants. Specifically, in field experiments we varied the richness of four entomopathogen species (two nematodes, two fungi) that attack herbivorous *Limonius californicus* larvae belowground. We show that the presence of entomopathogens increased wireworm mortality and subsequently increased plant productivity, but a single entomopathogen species, *Metarhizium brunneum*, had stronger effects than other species (*Beauveria bassiana*, *Heterorhabditis bacteriophora*, and *Steinernema carpocapsae*). Moreover, entomopathogen communities with *M. brunneum* consistently produced the strongest effects on prey mortality and plant productivity. Our results suggest that diverse entomopathogen communities increased prey infection more than any single species, indicating complementarity occurred. However, increased plant productivity appeared to be driven by species identify effects. Our study shows that the composition of entomopathogen communities had stronger effects on a pest species than species richness, suggesting that careful selection of entomopathogen species for biological control may be more impactful than promoting entomopathogen diversity.

1. Introduction

Across a diverse array of ecosystems, greater natural enemy diversity generally promotes increased mortality of prey resources (Griffin et al., 2013). Two mechanisms are generally believed to underlie these patterns: species complementarity and identity effects (Griffin et al., 2013; Crowder and Jabbour, 2014). Species complementarity occurs when natural enemy species feed on unique resources in space or time, whereby more diverse communities consume more overall resources by feeding across diverse niches (Finke and Snyder, 2008; Northfield et al., 2010). Identity effects arise when more species-rich communities contain the most impactful species by chance alone, leading to greater prey mortality (Crowder and Jabbour, 2014).

Studies examining the relationship between natural enemy diversity and prey mortality have largely focused on aboveground predator and prey communities (Griffin et al., 2013). These numerous studies have shown that increased predator diversity strengthens herbivore suppression through both complementarity and identity effects (Finke and Snyder, 2008; Northfield et al., 2010; Griffin et al., 2013). However, meta-analyses have revealed that species identity effects are often more impactful on prey mortality than complementarity effects (Griffin et al., 2013). Nevertheless, few studies have assessed whether increased diversity of entomopathogen species belowground has positive effects on the infection and mortality of prey species that feed on plant roots and seeds (Ramirez and Snyder, 2009; Jabbour et al., 2011). As plant productivity is strongly mediated by processes occurring in the soil, this represents a key knowledge gap in our broader understanding of the potential benefits of natural enemy biodiversity.

Here we assessed how diversity and composition of belowground entomopathogen communities affected a soil-dwelling herbivore (the sugarbeet wireworm, *Limonius californicus*) and the resulting productivity of wheat (*Triticum aestivum*) plants. Wireworms, the

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subterranean larvae of click beetles (Coleoptera: Elateridae), are a group of herbivorous soil-dwelling insects that feed on belowground plant tissues, causing economic damage across a wide geographic range (Traugott et al., 2015). Wireworms spend years in the soil, where they risk infection by soil-inhabiting pathogenic fungi and nematodes (Reddy et al., 2014; Traugott et al., 2015; Ensafi et al., 2018). However, although the species diversity of entomopathogens in the soil can vary dramatically across agroecosystems (Ramirez et al., 2009; Crowder et al., 2010), the potential effects of variation in entomopathogen species composition and diversity on wireworms remains largely unknown. Yet, as unique entomopathogen species differ in their resource acquisition strategies, there is reason to suspect that more diverse communities of entomopathogens might be more impactful than any single species. For example, the fungi *Beauveria bassiana* and *Metarhizium brunneum* rely on passive transmission of spores through direct contact with hosts, but the nematode *Heterorhabditis bacteriophora* actively searches for hosts (Lewis et al., 2006; Ramirez and Snyder, 2009). This suggests increased prey infection, and subsequent mortality, may be driven by complementarity effects when entomopathogens exist in diverse communities.

We examined these questions with a field experiment testing whether the diversity and/or composition of entomopathogens mediated effects on wireworms and wheat plants. Specifically, we assessed whether four soil-dwelling entomopathogen species, *B. bassiana*, *M. brunneum*, *H. bacteriophora*, and *Steinernema carpocapsae*, differed in their effects on wireworms and wheat plants when present alone or in diverse communities. Our substitutive experimental design allowed us to maintain constant entomopathogen densities and thus isolate species identity and complementarity effects. Overall, our results are applicable for management of wireworms in crop systems, and elucidate mechanisms driving entomopathogen diversity effects on prey.

2. Materials and methods

2.1. Experimental setup

Our study had four entomopathogen species: *B. bassiana*, *M. brunneum*, *H. bacteriophora*, and *S. carpocapsae*; each of these occur in crop systems of the Pacific Northwest United States (Reddy et al., 2014; Ensafi et al., 2018). Our focal plant, wheat (*T. aestivum*), is a common crop grown in the Pacific Northwest, and our focal herbivore, *L. californicus*, is a dominant wireworm species attacking wheat crops (Milosavljević et al., 2015, 2016a,b, 2017, 2019).

Our experiment was conducted at the Washington State University Orchard in Pullman, WA, USA. The site had a silt loam soil (8% sand, 68% silt, 24% clay) with a mean pH of 8.4 (SE = 0.02). Experimental units were plastic containers (60 × 45 × 40 cm) with holes (0.3 mm) that allowed for drainage but were small enough to prevent wireworm escape. These containers were installed in soil, with the top flush with the soil surface, and filled with excavated raw soil that was free from wireworms (see section *2.2* for more details). The experiment involved four treatments that varied in entomopathogen richness and composition: (1) control (eight replicates without entomopathogens), (2) one species (six replicates of each entomopathogen species present alone, 24 experimental units total), (3) two species (four replicates of each of the 6 unique pairs of entomopathogen species, 24 experimental units total), and (4) four species (24 replicates that included all four entomopathogen species). This design thus fully replicated all possible combinations of species composition and diversity within our community of four entomopathogen species, allowing us to assess complementarity and identity effects.

Wheat plants were sown directly into containers on 15 May 2015 and allowed to acclimate for 7 d before wireworms and entomopathogens were added. Each container received 8 wheat plants (var. 'Louise'), planted in 2 rows, with in-row and between-row spacing of 8 and 20 cm, respectively. 'Louise' is widely grown and is susceptible to wireworms (Milosavljević et al., 2019). Wireworms used were field collected within 2 wk of experiment initiation using bait traps and identified to species (Esser et al., 2015); all wireworms were *L. californicus*, a highly-damaging species in the field (Esser et al., 2015; Milosavljević et al., 2017, 2019). Wireworms were housed without food for 2 wk before introducing them to field mesocosms, after which 10 large larvae (> 8 mm in size) were assigned to each experimental mesocosm in the field.

Entomoathogens were: (1) *B. bassiana* (Mycotrol O, Emerald BioAgriculture, Lansing, MI, USA), (2) *M. brunneum* (Met52 EC, Novozymes Biologicals, Inc., Salem, VA, USA), (3) *S. carpocapsae* (NemaAttack, Arbico Organics, Oro Valley, AZ, USA), and (4) *H. bacteriophora* (NemaSeek, Arbico Organics, Oro Valley, AZ, USA). Monoculture treatments received fungi at a rate of 3.85×10^7 viable conidia/m^2, or nematodes at 3.1×10^6 nematodes/m^2. These rates were chosen as they infect ≈30% of *L. californicus* larvae in the field (Reddy et al., 2014). Two- and four species treatments received one-half or one-fourth these rates, respectively, so our design was substitutive, meaning we varied species composition and diversity without varying overall entomopathogen abundance. Entomopathogens were evenly applied to soil with a spray bottle. To enhance entomopathogen dispersal and survival, mesocosms were watered two hours prior to and after entomopathogen application. Watering frequency was regulated to keep soil saturation in each container similar to soil moisture observed in local wheat fields throughout the growing season. Each experimental unit was maintained until the wheat was mature and ready to harvest.

2.2. Soil sampling for wireworms and entomopathogens prior to experiments

To determine the species of wireworm(s) present at the study site, and their natural initial density, a total of 40 bait traps were deployed in a zig zag pattern. Traps were placed starting at the field edge and moving through the study area (with 2 m spacing), 10 d prior to experiments. Each trap consisted of a nylon stocking filled with 120 cm^3 of a wheat and corn seed mixture in a 50:50 ratio (Esser et al., 2015). Traps were submerged in water for 24 h prior to field deployment to start seed germination; germinating seeds produce and emit elevated amounts of CO_2 and attract wireworms (Johnson and Nielsen, 2012). Each trap was deployed in 20 cm deep hole in the ground, covered with sufficient soil, and flagged. All traps were retrieved after 8 d and transported to the laboratory, where they were examined for wireworms by hand. Wireworms are easily identifiable to species (Glen et al., 1943; Lanchester, 1946), providing an indication of whether the target herbivores are persisting in the field. We have used this approach to measure wireworms in several other studies (Milosavljević et al., 2015, 2016a,b, 2017, 2019). Target wireworms were not detected in bait traps collected from the study area prior to the experiments.

To measure the prevalence of nematodes and fungi at the study site, 100 g of soil was taken from each hole where wireworms were sampled, 10 d prior to the experiments, for a total of 40 soil samples. These samples were placed in plastic bag, marked, mixed to homogenize them, and assessed for soil infectivity in the laboratory by assigning each sample to an individual deli cup. After adding soil, 10 waxworm larvae (*Galleria mellonella*) (Sunshine mealworms, Silverton, OR) were introduced to each cup and kept for 7 d in the dark at room temperature, after which waxworms were recollected and mortality assessed. Waxworms are a good indicator species for entomopathogen persistence in the soil as they are highly susceptible to both nematodes and fungi (Ramirez et al., 2009). Waxworms that are killed by entomopathogens are easily identified, indicating whether the target species are present in the field. Target entomopathogens were not detected in soil samples collected from the study area prior to the experiments.

2.3. Data collection. Plants were harvested on 2 October 2015, and six productivity measures were taken: (i) aboveground biomass, (ii)

belowground biomass, (iii) number of heads, (iv) harvest index, (v) seed weight, and (vi) seed viability. All plants were harvested at the base, after which the number of heads were counted. The aboveground matter was then dried in an oven at 100 °C for 48 h and weighed to record aboveground biomass. Harvest index was the ratio of head weight to biomass (Yadav et al., 2002). All produced seeds were weighed. Each seed was germinated on blotter paper, moistened with tap water, and placed in a growth chamber at 20 °C for 7 d, after which seed viability was assessed using standardized procedures from the Association of Official Seed Analysts (AOSA, 2000). To record root biomass, we extracted root cores from the soil with soil-sorting sieves and washed residual soil off the roots carefully with tap water; all roots were then oven-dried at 100 °C for 48 h before measuring dry mass.

To indirectly measure entomopathogen persistence in the soil, we used waxworm mortality as a proxy. Every 4 wk during the experiment (22 May, 19 June, 17 July, 14 Aug, 11 Sep, and 9 Oct), 10 waxworms were placed in the soil of each mesocosm in a black mesh bag made from fiberglass window screen (diameter 0.3 mm). These bags were then covered with excavated soil. Waxworms were left in soil for 72 h, after which they were removed and placed on filter paper in petri dishes in the laboratory. Mortality was assessed 1 wk later. At the end of the experiment, wireworms were extracted each mesocosm using soil sieves. Dead larvae manifested no motion after being introduced to a light source for 10 s; other larvae were counted as survived.

2.3. Data analysis

All analyses were conducted in R v. 3.6.1 (R Core Team, 2019). We tested effects of entomopathogens on eight responses: (i) aboveground biomass, (ii) belowground biomass, (iii) number of heads, (iv) harvest index, (v) seed weight, (vi) seed viability, (vii) wireworm mortality, and (viii) waxworm mortality; separate analyses were conducted for each response. In the first set of analyses, we used Welch's *t*-tests (due to unequal sample size and variance) to assess whether entomopathogen treatments (pooled across all treatments) affected each response compared to controls without entomopathogens; these analyses determined overall effect of entomopathogens without considering diversity or identity effects. Second, we used linear regressions to determine whether the number of entomopathogen species (pooled across all treatments with the same number of species) affected each response; these analyses determined overall effects of entomopathogen diversity but not the mechanisms driving diversity effects. In regressions, seed viability, wireworm mortality, and waxworm mortality were treated as binary response variables, and all other responses were treated as continuous variables. Waxworm mortality was also treated as a repeated measures mixed analysis (as 6 assessments were made during the experiment), with mesocosm as a random effect.

We assessed if species complementarity or identity effects mediated entomopathogen diversity effects on wireworms and plants. For each unique diverse entomopathogen assemblage j we measured non-transgressive (D_T) and transgressive (D_{max}) over-yielding as:

$$D_T = (O_j - E_j)/E_j \quad (1)$$

$$D_{max} = (O_j - M_j)/M_j \quad (2)$$

where O_j is observed plant productivity or wireworm mortality in diverse community j, E_j is the expected productivity or mortality in diverse community j (the average productivity or mortality across each entomopathogen species present), and M_j is the productivity or mortality associated with the most impactful entomopathogen species in diverse community j (in our case the entomopathogen species that promoted the highest wheat productivity, or caused the greatest wireworm mortality) (Petchey, 2003; Milosavljević et al., 2016b). Separate analyses were conducted for each plant productivity metric and for wireworm mortality.

Non-transgressive overyielding indicates that plant productivity or wireworm mortality in a diverse entomopathogen community differed from average productivity when entomopathogen species were present singly; D_T thus indicates the net impact of diversity (Petchey, 2003). If D_T values are above 0, it indicates diverse entomopathogen communities increased productivity or mortality more than entomopathogen species present alone; D_T values < 0 indicate diverse communities had reduced effects on productivity or mortality than single species. Transgressive overyielding, D_{max}, occurs when effects of a diverse entomopathogen community exceeds that of the most impactful entomopathogen species. In this case, if D_{max} values are above 0, it indicates that diverse entomopathogen communities increased plant productivity or wireworm mortality more than the single most impactful entomopathogen species present alone; D_{max} values below 0 indicate that the single most impactful entomopathogen species had greater effects on plant productivity or wireworm mortality than a diverse entomopathogen community.

If D_T and D_{max} are both positive, it indicates a positive effect of entomopathogen diversity driven by species complementarity. If D_T is positive, but D_{max} is zero or negative, it would suggest that the most impactful species exerted stronger effects than the diverse community (i.e., an identity effect). However, such measurements are only suggestive of mechanisms driving species diversity effects, and may fail to capture diversity effects when non-linear relationships between species abundance and ecosystem services occur (Pillai and Gouhier, 2019). We calculated D_T and D_{max} for each plant productivity metric and wireworm mortality. We used one-sample *t*-tests to assess whether these values, calculated across all of the unique two- and four-species entomopathogen communities, differed significantly from 0. This allowed us to detect if over-yielding occurred, and whether species identity or complementarity played a dominant role in wireworm communities (Petchey, 2003; Milosavljević, et al., 2016b).

3. Results

3.1. Effects of entomopathogens on wireworms and waxworms

Entomopathogen treatments had greater wireworm mortality than controls ($t = 7.13$, df = 12.8; $P < 0.0001$), and greater entomopathogen species richness promoted wireworm mortality ($Z = 10.7$, $P < 0.0001$) (Fig. 1A). Diverse communities increased mortality compared to the expected mortality for species in those communities ($D_T > 0$; Table 1), and non-transgressive over-yielding occurred ($D_{max} > 0$, Table 1), indicating that species complementarity was the primary mechanism driving positive effects of entomopathogen diversity on wireworm mortality. However, different entomopathogen species varied in their effects on wireworm mortality. The most impactful entomopathogen species was *M. brunneum*, and communities containing this species consistently produced higher wireworm mortality than communities without it (Fig. 1B).

Entomopathogen treatments also had significantly higher waxworm mortality than controls without entomopathogens ($Z = 4.29$, $P < 0.0001$); this effect was consistent over time ($Z = 0.48$, $P = 0.64$). Increasing entomopathogen richness promoted greater waxworm mortality ($Z = 4.81$, $P < 0.0001$), and this effect increased over time ($Z = 3.11$, $P = 0.0019$) (Fig. 2).

3.2. Effects of entomopathogens on plant productivity

The presence of entomopathogens increased plant aboveground and belowground biomass, but had no significant effects on the number of heads, harvest index, seed weight, and seed viability (Table 2). However, increasing entomopathogen species richness increased every plant productivity metric except harvest index (Table 2, Fig. 3A-F). Diverse entomopathogen communities increased plant productivity more than expected for three plant metrics, aboveground biomass, number of heads, and seed weight (D_T values > 0), but increased belowground

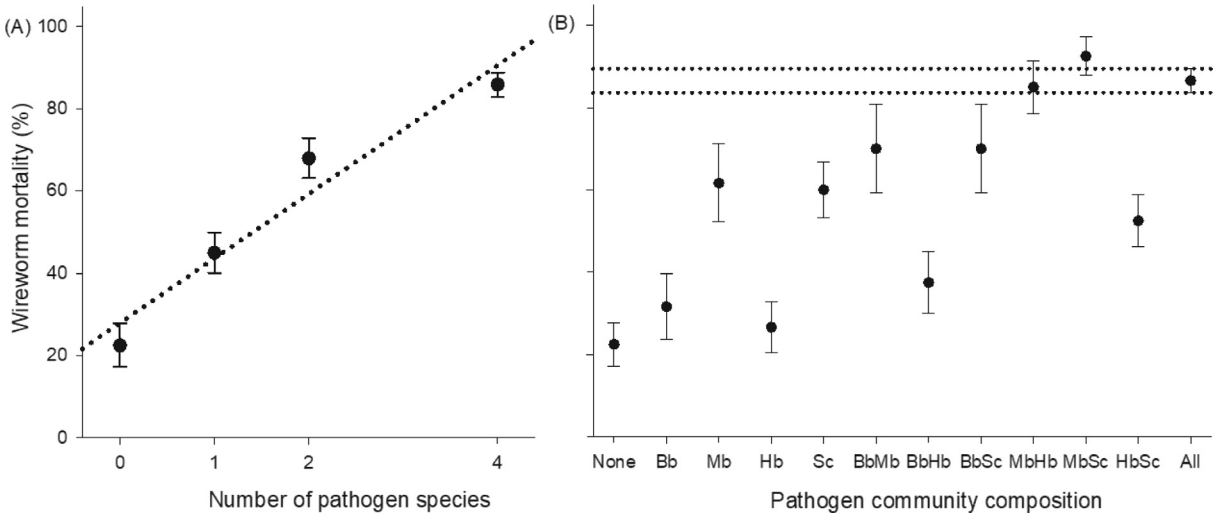

Fig. 1. Effects of (A) pathogen species richness and (B) pathogen community composition on wireworm mortality. In A, values for each level of pathogen richness were pooled, and the best-fit regression line is shown. In B, the average mortality with each unique pathogen community composition is shown, where the dashed lines indicate mortality in the most diverse community with all four pathogen species (Bb = *Beauveria bassiana*; Mb = *Metarhizium brunneum*; Hb = *Heterorhabditis bacteriophera*; Sc = *Steinernema carpocapsae*).

Table 1
Results of non-transgressive (D_T) and transgressive (D_{max}) over-yielding on multiple wheat productivity metrics from one-sample *t*-tests (*t* values with 47 degrees of freedom, and associated *P* values are shown). Values of D_T greater than 0 indicate that diverse communities of pathogens performed better than expected, based on the average productivity in monocultures. Values of D_{max} greater than 0 indicate diversity effects were driven by species complementarity, values less than 0 indicate that diversity effects were driven by species identity.

Response	D_T		D_{max}	
	t_{47}	*P*	t_{47}	*P*
Wireworm mortality	11.5	< 0.0001	6.80	< 0.0001
Aboveground biomass	3.62	0.0007	−4.10	0.0002
Belowground biomass	−2.50	0.02	−13.1	< 0.0001
Number of heads	3.36	0.0016	−2.5	0.015
Harvest index	1.18	0.24	−4.19	0.0001
Seed weight	2.43	0.019	−4.59	< 0.0001
Seed viability	3.73	0.0005	−2.30	0.026

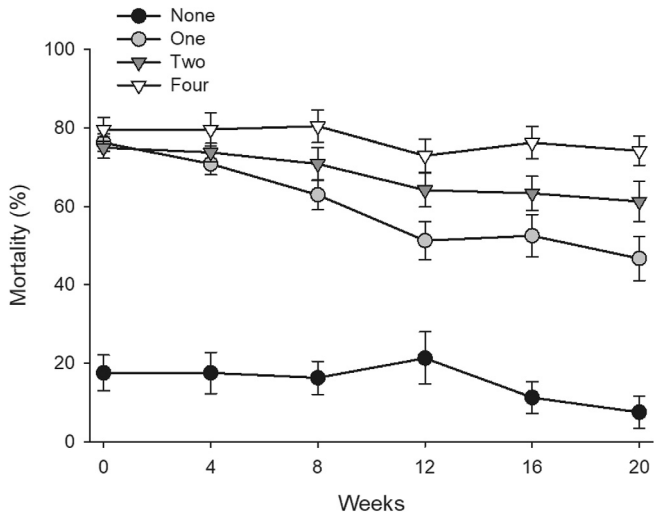

Fig. 2. Effects of pathogen species richness on waxworm mortality. Values for each level of pathogen richness were pooled, and the best fit regression is shown.

Table 2
Effects of pathogen presence and species richness on wheat productivity metrics: (i) aboveground dry mass, (ii) belowground dry mass, (iii) number of heads, (iv) harvest index, (v) seed weight, and (vi) seed viability. Results shown are from Welch's *t*-tests testing effects of pathogen presence, linear regressions testing effects of pathogen species richness for each response variable except seed viability (*t*-tests), and logistic regression models testing effects of pathogen richness on seed viability (*Z*-tests).

Response	Pathogen presence			Species richness		
	t	df	*P*	*Estimate*	t_{78} *or Z*	*P*
Aboveground biomass	−2.72	9.09	0.023	36.1	3.63	0.0005
Belowground biomass	−3.35	12.8	0.0053	25.0	5.42	< 0.0001
Number of heads	−1.76	8.33	0.11	1.47	2.14	0.035
Harvest index	0.082	8.00	0.94	0.012	0.78	0.43
Grain weight	1.52	8.45	0.16	3.67	2.38	0.020
Seed viability	−1.81	8.62	0.10	0.080	3.35	0.0008*

biomass less than expected based on the average of the monocultures (D_T value < 0) (Table 1). Effects of entomopathogen diversity for all plant productivity metrics were driven by species identity rather than complementarity effects (D_{max} < 0 for all metrics) (Table 1). As with wireworm mortality, *M. brunneum* was the most impactful single entomopathogen species on promoting plant productivity, and diverse communities with this species consistently performed better than communities without it (Fig. 4A-F).

4. Discussion

Our study supports the idea that both the composition of entomopathogen communities and species richness may be important for biological control (Griffin et al., 2013). While the presence of entomopathogens increased wireworm mortality and in turn increased plant productivity, a single species, *M. brunneum*, produced stronger effects than *B. bassiana*, *H. bacteriophora*, and *S. carpocapsae*. Moreover, the greater impact of *M. brunneum* was seen across mesocosms that varied in community structure. The addition of *M. brunneum* to entomopathogen communities substantially increased the net number of prey that were killed, and subsequently increased plant productivity, in species-rich communities. Because the addition of *M. brunneum* suppressed *L. californicus* feeding and survival, and subsequently increased plant productivity, this functionally unique species has the potential to

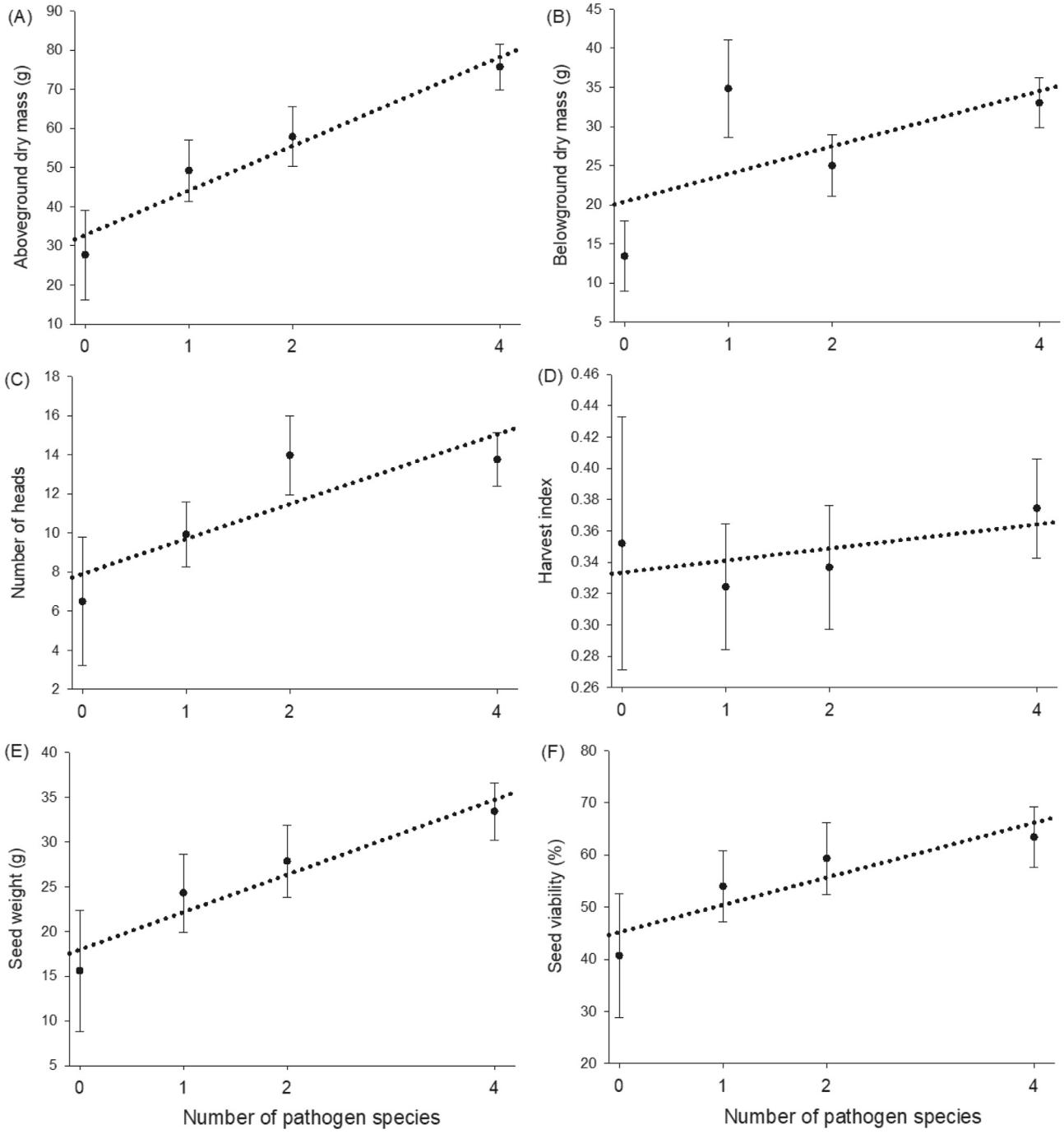

Fig. 3. Effects of pathogen species richness on (A) aboveground dry mass, (B) belowground dry mass, (C) number of heads, (D) harvest index, (E) seed weight and (F) seed viability. In each panel, values show the mean (± SE) for each level of diversity, pooled across treatments with the same richness, and the best-fit regression line.

mitigate the costs associated with wireworm feeding and should be explored by biological control practitioners.

The concurrent application of fungi and nematodes for biological control of soil-dwelling herbivores have produced inconsistent results that depend on the entomopathogen species, the host species, and the timing of applications, and include synergisms (Ansari et al., 2006), additive effects (Jabbour et al., 2011), and antagonism (Shapiro-Ilan et al., 2004). Our study found the effects of multiple entomopathogen species were non-additive, where more diverse entomopathogen communities produced greater wireworm mortality than any single species. This suggests that complementarity among entomopathogens promoted wireworm mortality.

Previous work in entomopathogen-prey systems shows different nematode species use different host finding strategies and occupy different soil depths (Neumann and Shields, 2006), encouraging their ability to partition resources. Nematodes that use a cruising foraging strategy are often better at locating immobile hosts deep in the soil profile, while sit-and-wait and sit-and-pursue predators are better at finding mobile hosts near soil surfaces (Bal and Grewal, 2015). Greater wireworm mortality in diverse communities may have resulted from complementarity between cruising and passive species, or species that occupied different soil depths. For example, *H. bacteriophora* is a cruising species that infects prey deeper in the soil surface compared to the sit-and-wait nematode *S. carpocapsae* (Lewis et al., 2006). The

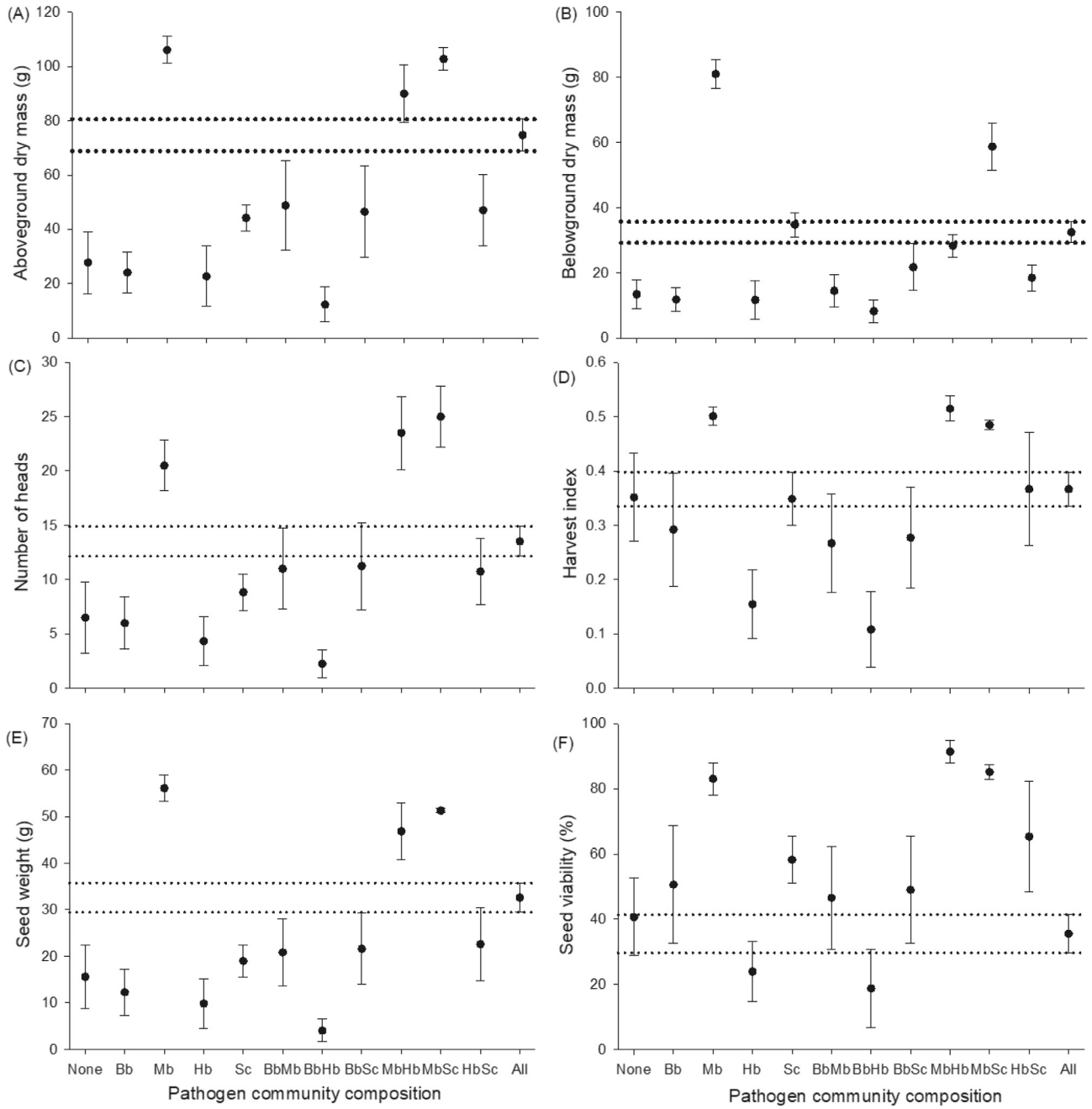

Fig. 4. Effects of pathogen composition on (A) aboveground dry mass, (B) belowground dry mass, (C) number of heads, (D) harvest index, (E) seed weight, and (F) seed viability. In each panel, the mean value for each metric (± SE) with each unique pathogen community composition is shown, where dashed lines indicate values from the most diverse community with all four pathogen species (Bb = *Beauveria bassiana*; Mb = *Metarhizium brunneum*; Hb = *Heterorhabditis bacteriophera*; Sc = *Steinernema carpocapsae*).

passive fungal species *M. brunneum*, which has spores near the soil surface, may have also been complementary to the nematode species on affecting wireworms attracted to plant roots (Milosavljević et al., 2017).

Although we observed complementary effects among entomopathogens on wireworm mortality, we found that plant productivity appeared to be primarily driven by entomopathogen species identity effects, with the greatest productivity in communities with *M. brunneum*. The lack of correlation between effects on wireworm mortality, and plant productivity, strongly suggests that indirect effects of entomopathogens, such as effects on wireworm feeding rates, may have important implications (Milosavljević et al., 2016b). Similarly, neonicotinoid insecticides, which are widely used for wireworm management, typically fail to kill wireworms but can prove beneficial for plant production by decreasing feeding rates (Esser et al., 2015; Milosavljević et al., 2019). However, it is also possible that we failed to capture potential complementarity effects among entomopathogens on plant productivity due to assumptions of the D_t and D_{max} metrics. While these metrics are widely used in the ecological literature, they may fail to capture potential complementarity effects if effects of species abundance on ecosystem services are non-linear (Pillai and Gouhier, 2019).

Another explanation of why some indicators of plant productivity were greatest where M. brunneum was applied relates to the potential direct effects of this entomopathogen species on plant physiology and vigor (Behie and Bidochka, 2014; Russo et al., 2015; Sánchez-Rodríguez et al., 2018). In addition to their direct negative effects on soil

herbivores through soil application, *M. brunneum* may also play an important role in reducing herbivory following their colonization of plants as endophytes (Vidal and Jaber, 2015). Plant colonization by *M. brunneum* has also been reported to boost the defense mechanisms of the plant that produce secondary metabolites and root biomass, inducing yield gains that could compensate for potential effects of wireworm damage (Jaber and Ownley, 2018).

While our results show that the inclusion of a superior entomopathogen (M. brunneum) may drive the most effective management of *L. californicus*, our experiments were conducted at a single level of wireworm abundance. Studies of entomopathogen diversity and prey mortality show that when prey abundance is low, competition for cadavers intensifies, and effects of diversity may be minimal because there are too few resources available to consumers. In such cases, the most effective species is often the one that develops fastest, or is the most generalist (Helmberger et al., 2017). Fungi and nematodes can colonize different parts of the same cadaver (Tarasco et al., 2011), yet this decreases resources available to inferior competitors, and limits their potential to persist (Blanco-Pérez et al., 2019). Other studies show that *M. anispoliae* excludes nematodes such as *S. carpocapsae* and *H. bacteriophora* after several days of infection when host density is low (Ansari et al., 2006). However, with more wireworms or host diversity, we may have observed different effects of entomopathogen species richness.

Benefits of entomopathogen diversity can also be affected by soil conditions (Helmberger et al., 2017). Soil texture and organic media often affect nematode efficacy, which move by using water films around soil particles (Villani and Wright, 1990). Greater movement through the soil by cruisers such as *H. bacteriophora*, and increased prey infection, have been seen in sandy soils; in fine silt and clay soils cruising is restricted by small particle size (Barbercheck, 1992; Ensafi et al., 2018). In contrast, ambush-foraging *S. carpocapsae* can seize their hosts easier in fine textured and organic soils by waiting for their prey along cracks and large pore spaces formed by plant roots (Griffin, 2012). Again, this could explain stronger effects of *S. carpocapsae* compared to *H. bacteriophora* in silty soils in our study. It is possible, however, that a broader range of soil conditions in the field may drive greater complementarity among entomopathogens not seen in our experiments that were conducted in a single soil type.

The effect of soil texture may not be as important for fungal species because they are non-motile. Nevertheless, studies show that efficacy and persistence of soil fungi are generally lower in sandy loam soils, and increase with higher clay content (Brownbridge, 2006; Scheepmaker and Butt, 2010). Once leached to a depth beyond that inhabited by host larvae, fungal propagules are no longer effective against target wireworms that feed on plant roots, and there is lower chance of inocula levels increasing. Our results, however, tentatively support the idea that some species or strains (e.g., M. brunneum) perform better in certain soils than others, so the plant response can be greater. Indeed, notable in our data was a particularly dramatic increase in host mortality in diverse communities with M. brunneum. In contrast, our results show that *B. bassiana* activity decreased over time (with waxworms as the focal host), potentially diminishing their impact. In line with these findings, M. brunneum are more often associated with fine textured soils and may persist longer in the absence of hosts than *B. bassiana* (Quesada-Moraga et al., 2007). Our field data similarly confirmed the persistence of M. brunneum for the duration of our experiments, without being affected by dry soil conditions during summer. This indicates that M. brunneum may be more suitable for prophylactic application than B. bassiana.

Non-chemical management tactics for wireworms have received scant attention, despite the pervasive use of neonicotinoids as the sole control measure (Furlan and Kreutzweiser, 2015). Studies show that entomopathogens such as nematodes and fungi can suppress wireworm populations in the lab (Eckard et al., 2014; Ensafi et al., 2018; Sandhi et al., 2020), although results are more variable in the field (Ansari et al., 2009; Reddy et al., 2014; Sharma et al., 2020). Our data suggest that parasitic fungi and nematodes may improve wireworm control in the Pacific Northwest United States, although the species of entomopathogens may matter more than diversity. However, whether these biological control agents provide adequate control under realistic field conditions in the region remains unknown. Thus, efforts should be made to investigate whether these putative agents can provide control of wireworms when used in combination with, or as a replacement for, seed-applied neonicotinoids (Shapiro-Ilan et al., 2006; Reddy et al., 2014).

By evaluating economic costs of each treatment, the viability of entomopathogens for use by commercial growers can be determined (Milosavljević et al., 2019). This is critical to promote adoption of biological control in the region. There is also a need to screen more entomopathogen species or strains (Sandhi et al., 2020), particularly species collected from cereal fields inhabited by wireworms, to determine their efficacy under specific wireworm infestations (and testing various hosts) and defined environmental conditions (Kleespies et al., 2013). Such strains may have significant advantages over commercially purchased entomopathogens. Overall, however, we suggest that the careful selection of entomopathogen species for biological control may provide increased flexibility for growers while also increasing profitability and sustainability.

Author contributions

All author designed the study, IM and ADE conducted the experiments, IM and DWC analyzed the data, and IM and DWC wrote the paper with edits from ADE and AR.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2020.104317.

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