

LETTER

Soil fungal pathogens and the relationship between plant diversity and productivity

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Abstract

One robust result from many small-scale experiments has been that plant community productivity often increases with increasing plant diversity. Most frequently, resource-based or competitive interactions are thought to drive this positive diversity–productivity relationship. Here, we ask whether suppression of plant productivity by soil fungal pathogens might also drive a positive diversity–productivity relationship. We created plant assemblages that varied in diversity and crossed this with a \pm soil fungicide treatment. In control (non-fungicide treated) assemblages there was a strong positive relationship between plant diversity and above-ground plant biomass. However, in fungicide-treated assemblages this relationship disappeared. This occurred because fungicide increased plant production by an average of 141% at the lower ends of diversity but boosted production by an average of only 33% at the higher ends of diversity, essentially flattening the diversity–productivity curve. These results suggest that soil pathogens might be a heretofore unappreciated driver of diversity–productivity relationships.

Keywords

Diversity–productivity, native plant diversity, plant productivity, soil pathogens.

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INTRODUCTION

Research on how biodiversity influences ecosystem function has yielded one very robust result. At small spatial scales, community productivity generally increases with the number of species in local communities (Hector *et al.* 1999; Tilman *et al.* 2001; Hooper *et al.* 2005; Balvanera *et al.* 2006; Cardinale *et al.* 2006). Two non-mutually exclusive phenomenological processes have been widely discussed as driving this relationship. The first is complementarity, whereby more diverse assemblages produce more biomass than what is expected based on the performance of individual species in monoculture (Loreau & Hector 2001). The second is the ‘sampling’ or ‘selection’ effect (Aarssen 1997; Huston 1997; Loreau & Hector 2001), whereby a positive diversity–productivity relationship is driven by a particularly productive species.

The biological mechanisms that have most often been attributed to producing complementarity and/or the selection effect are either niche partitioning or strong competitive interactions. Greater niche partitioning at higher levels of diversity can lead to gains in productivity because as more niches become filled there is greater efficiency of resource uptake, which fuels greater cumulative production of biomass in an assemblage (Tilman *et al.* 2001; Cardinale *et al.* 2007; Fargione *et al.* 2007; Fornara & Tilman 2009). Alternatively, strong competitive interactions can drive greater production at increasing levels of diversity if one particularly productive species is a superior competitor and the probability of an assemblage containing this species increases with increasing diversity (Roscher *et al.* 2009). While most of the work on biodiversity–ecosystem function has evaluated these two drivers of positive diversity–productivity relationships (Cardinale *et al.* 2006, 2007), other possible biological mechanisms that might produce this relationship have received less attention.

We investigated whether the diversity–productivity relationship might be driven by soil fungal pathogens as opposed to more widely studied resource-based competitive processes. There are several reasons why soil pathogens might play a larger-than-thought role in creating the diversity–productivity relationship. First, negative feedbacks between plants and soil microbes are common and can importantly influence plant productivity (Van der Putten *et al.* 1993; Bever 1994; Packer & Clay 2000; Klironomos 2002; Kulmatiski *et al.* 2008). Second, the negative effects of soil pathogens can be species-specific and density-dependent (Janzen 1970; Connell 1971; Van der Putten *et al.* 1993; Mills & Bever 1998; Packer & Clay 2000; Klironomos 2002; Bell *et al.* 2006; Kardol *et al.* 2006). As such, soil pathogens might have larger negative effects on plants growing at low vs. high diversity because the relative abundance of any particular plant host is generally greater in low than high-diversity assemblages. If this were the case, soil pathogens could create a positive relationship between diversity and productivity by strongly reducing the biomass of plants in low-diversity mixtures while having less negative impacts on plants in high-diversity assemblages. This predicts that experimentally suppressing soil fungal pathogens should eliminate the positive diversity–productivity relationship by differentially enhancing productivity at the low end of diversity (Fig. 1d).

To test this prediction, we created native plant assemblages that varied in different components of diversity. We crossed each unique diversity treatment with a \pm soil fungicide treatment to test the influence of pathogenic soil fungi on community productivity. Our goal was to explore whether fungicide application fundamentally altered the relationship between native plant diversity and productivity.

METHODS

Experimental set-up

We created plant assemblages that were composed of native perennial species that commonly co-occur in grasslands of western Montana (Table 1). Our experiment consisted of 14 unique plant assemblages that varied in different components of diversity (Tables 1 and 2). Assemblages consisted of mixes of 2, 5, 6, 7, 8, 10, 11, 12, 14 or 16 native species. Within particular levels of species richness, functional richness (based on rooting morphology and phenology; Table 1) varied (Table 2). Each diversity treatment was crossed with a \pm soil fungicide treatment in a full-factorial design with each combination replicated three times (14 diversity treatments \times 3 replications \times \pm fungicide = 84 plots total).

In creating our diversity treatments, our original goals were to: (1) determine the relative importance of species versus functional richness in driving a positive diversity ecosystem function relationship and (2) examine the effects of species and functional richness independently of species identity. To accomplish goal 1, we created some plant assemblages where species richness was held constant but functional richness increased (and *vice versa*). In this scenario, we would be able to test whether the biomass of a mixture at constant species richness increased with increasing functional richness or *vice versa*. To accomplish goal 2, in all cases where a diversity treatment contained only a subset of species from a particular functional group, species from that functional group were randomly assigned to those particular diversity treatments so that species identity could be de-coupled from species/ functional group richness. However, as the experiment proceeded through time, many assemblages lost species or functional groups such that the actual species and functional richness at the end of the experiment (i.e., ‘realized’ species and functional richness) varied from planted richness (Fig. 2a; Table 2). As a result, we ultimately lacked replicated assemblages with constant levels of species richness across which functional richness varied (and *vice versa*).

In May 2005 (and supplemented again in 2006), we transplanted 4-month-old seedlings grown from locally collected seed into 84 different 1.5 m \times 1.5 m plots in a fallow field at Fort Missoula, Missoula County, USA. This field contained intact soil that was not tilled or altered prior to the set-up of this experiment. Plots were arranged in three blocks, with blocks separated from each other by 4 m and plots within blocks separated by 2 m. Each diversity treatment was randomly assigned to receive either a soil fungicide drench or water (as a control for water in the fungicide soil drench). We first applied fungicide in October 2007 and reapplied it in April and October 2008, and in May 2009. Treated plots received a mix of two fungicides, Cleary’s 3336 (Cleary Chemical Corporation, Dayton, NJ, USA) and Ridomil Gold EC (Syngenta Corporation, Greensboro, NC, USA). The non-target effects of these fungicides are not well established, but Cleary’s 3336 (active ingredient: thiophanate ethyl) is a systemic, broad-spectrum fungicide that suppresses pathogenic fungi in the genus *Fusarium*. Ridomil Gold is also a systemic fungicide (active ingredient is mefenoxam, a synthetic isomer of metalaxyl) that controls diseases caused by Oomycete fungi, particularly Pythaceous fungi. Ridomil Gold has been successfully used in other ecological experiments (Bell *et al.* 2006). Thiophanate ethyl can reduce arbuscular mycorrhizal fungi (AMF) root colonization if applied at a relatively high frequency (3-week intervals through the growing) although this reduction in mycorrhizal root infection had no significant effect on shoot biomass for a C₃ grass and also had no phytotoxic effects (Wilson & Williamson 2008). Mefenoxam has low toxicity to

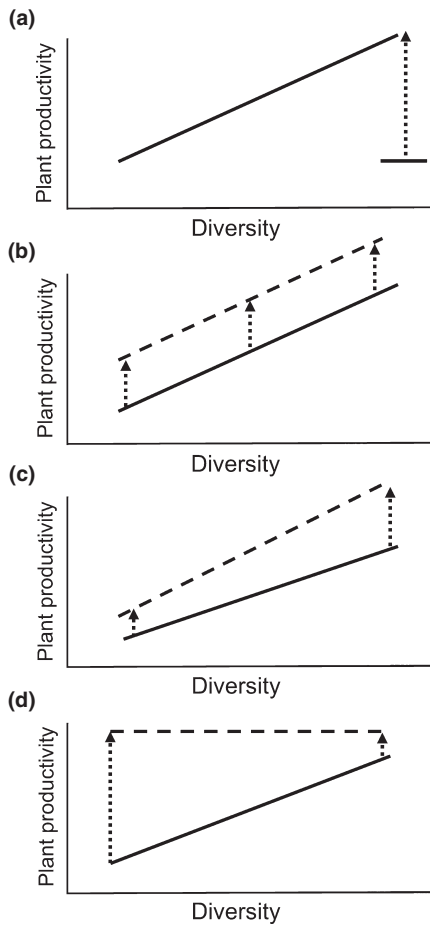


Figure 1 Conceptualized relationship between plant diversity and productivity. (a) Positive diversity–productivity relationship (shown as linear rather than curvilinear form for simplicity) with dashed arrow indicating that most work attempting to explain the positive diversity–productivity relationship has sought explanations for the increase in productivity at high levels of diversity. (b, c and d) Alternative hypotheses for how the diversity–productivity relationship might be altered by suppression of soil pathogens (dashed line). Please note that predictions from these hypotheses might be altered depending on how individual species differ in inherent susceptibility to pathogen attack. (b) Outcome if generalist soil pathogens have similar suppressive effects on community productivity across all levels of diversity. (c) Outcome if a selection effect drove the positive diversity–productivity relationship in the presence of pathogens (solid line). This would imply that a particularly productive species was dominant at high diversity, but absent within low-diversity assemblages. If this was the case, and the relative abundance of a productive species at high diversity was greater than the relative abundance of any one species within low-diversity assemblages, then density-dependent pathogen attack might have greater impacts at high vs. low diversity. (d) Outcome if pathogens act in a species-specific density-dependent manner and species grow at higher relative abundance at low vs. high diversity.

organisms other than fungi, and does not have strong inhibitory effects on AMF in agricultural systems (Afeke *et al.* 1990; Seymour *et al.* 1994).

Fungicide was delivered to each plot as a 3-L soil drench composed of a water–fungicide mix (1 g Cleary’s 3336 per litre H₂O and 0.117 mL Ridomil Gold EC per litre H₂O). The fungicide mix was applied with a sprayer attached to a pressurized tank. We calibrated the application rate (by timing how long it took to deliver 1.5 L of liquid) and sprayed each plot for the same amount of time. Control plots received only 3 L of water. Assemblages were weeded continuously throughout each growing season to maintain pre-determined levels of species and functional richness. Continuous removal of weed seedlings when tiny reduced disturbance due to weeding.

In May 2009, we recorded the ‘realized’ species richness of each plot by recording the identity of all species rooted within a 1-m² quadrat placed in the centre of each plot. In mid-July 2009, we harvested all plants rooted within a 0.75 m × 0.75 m quadrat placed in the centre of each plot. Plants were cut at ground level, sorted to species, dried to a constant weight at 60 °C and then weighed. Two of the spring ephemerals in the 16 species mixes, *Dodecatheon pulchellum* and *Lewisia rediviva*, had senesced prior to our

Table 1 Individual species used in experimental mixtures with corresponding functional group coding used in Table 2. Functional groups are grasses (G₁, species a–c), early flowering forbs that branch with short rhizomes, stolons or at the root crown (F₁, species a–c), mid-season forbs with spreading rhizomes (F₂, species a–c), mid-season forbs with woody root crowns (F₃, species a–c), late season forbs with deep tap roots (F₄, species a, b) and very early flowering ephemeral forbs with a shallow tap root (F₅, species a, b)

Species	Code
<i>Festuca idaboensis</i>	G _{1a}
<i>Koeleria macrantha</i>	G _{1b}
<i>Poa sandbergii</i>	G _{1c}
<i>Geum triflorum</i>	F _{1a}
<i>Antennaria rosea</i>	F _{1b}
<i>Penstemon procerus</i>	F _{1c}
<i>Arnica sororia</i>	F _{2a}
<i>Achillea millefolium</i>	F _{2b}
<i>Monarda fistulosa</i>	F _{2c}
<i>Penstemon wilcoxii</i>	F _{3a}
<i>Potentilla arguta</i>	F _{3b}
<i>Gaillardia aristata</i>	F _{3c}
<i>Aster ericoides</i>	F _{4a}
<i>Artemisia frigida</i>	F _{4b}
<i>Dodecatheon pulchellum</i>	F _{5a}
<i>Lewisia rediviva</i>	F _{5b}

Table 2 Composition of experimental assemblages (see coding in Table 1). Where specific species identity is not shown (i.e., where there is an ‘x’ or ‘y’ subscript denoting species identity), for each replicate of that treatment the species was drawn at random from the pool of two or three species from that particular functional group

Assemblage composition	SR	FR	Realized SR-C	Realized SR-F
G _{1a} G _{1b}	2	1	2, 2	2, 2
G _{1a} G _{1b} F _{1a} F _{1b} F _{1c}	5	2	4, 5	4, 4, 5
G _{1a} G _{1b} F _{1x} F _{1y} F _{2x}	5	3	3, 5	3, 5
G _{1a} G _{1b} F _{1x} F _{1y} F _{2x} F _{2y}	6	3	3, 4, 6	4, 4, 6
G _{1a} G _{1b} F _{1x} F _{2x} F _{3x} F _{4x}	6	5	4, 4, 6	3, 5, 5
G _{1a} G _{1c} F _{2x} F _{3x} F _{4x} F _{4y}	7	4	5, 5, 6	4, 6, 7
G _{1a} G _{1b} F _{2x} F _{2y} F _{3x} F _{3y} F _{4x} F _{4y}	8	4	7, 7, 8	6, 6, 6
G _{1a} G _{1b} F _{1a} F _{1b} F _{1c} F _{2a} F _{2b} F _{2c} F _{3x} F _{3y}	10	4	7, 8, 9	8, 9, 9
G _{1a} G _{1b} F _{1x} F _{1y} F _{2x} F _{2y} F _{3x} F _{3y} F _{4x} F _{4y}	10	5	5, 6, 9	6, 9, 10
G _{1a} G _{1b} F _{1a} F _{1b} F _{1c} F _{2a} F _{2b} F _{2c} F _{3a} F _{3b} F _{3c}	11	4	7, 8, 9	8, 9, 9
F _{1a} F _{1b} F _{1c} F _{2a} F _{2b} F _{2c} F _{3a} F _{3b} F _{3c} F _{4a} F _{4b}	11	4	9, 9, 11	5, 6, 8
G _{1a} G _{1b} F _{1a} F _{1b} F _{1c} F _{2a} F _{2b} F _{2c} F _{3a} F _{3b} F _{3c} F _{4b}	12	5	4, 9, 10	7, 7, 9
G _{1a} G _{1b} G _{1c} F _{1a} F _{1b} F _{1c} F _{2a} F _{2b} F _{2c} F _{3a} F _{3b} F _{3c} F _{4a} F _{4b}	14	5	10, 11, 13	4, 11, 12
G _{1a} G _{1b} G _{1c} F _{1a} F _{1b} F _{1c} F _{2a} F _{2b} F _{2c} F _{3a} F _{3b} F _{3c} F _{4a} F _{4b} F _{5a} F _{5b}	16	6	11, 13, 15	11, 13, 14

Realized SR-C and realized SR-F are the actual species richness in each replicate control and fungicide-treated plot respectively, at the end of the experiment.

harvest and therefore could not be weighed (even though these were included in calculations of realized species richness). However, these small-statured species occurred at low density and thus their cumulative biomass per subplot was likely very small. Furthermore, the abundance of these two species did not differ substantially between fungicide-treated and control plots.

Effects of fungicide on infection of plant roots by pathogenic lesions

We sampled a subset of plots to determine how fungicide application influenced infection of roots by both pathogenic lesions and AMF. In October 2009, we sampled soil from 12 control plots (realized species richness of sampled assemblages was 2–15) and 12 fungicide-treated plots (realized species richness = 2–14). Three randomly located soil core samples were extracted per plot (0–15 cm deep) and were combined to form one composite sample. These were overnight shipped to the laboratory of J. Klironomos, where roots from samples were examined for parasitic/ pathogenic fungi. All roots were extracted from the soil samples, washed free of soil, cut into 5 cm fragments and placed in 50% ethanol. Roots were then cleared with 10% KOH and assessed at 100× magnification (at 150 intersects along the root) for any necrotic lesions on their outer surface. Roots were then stained with Chorazol Black E (Brundrett *et al.* 1984) and assessed at 250× magnification (at 150 intersects along the root) for per cent

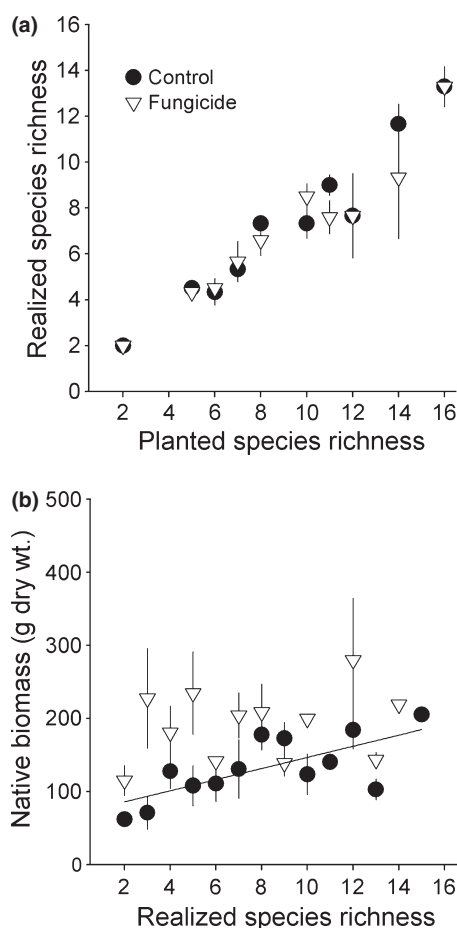


Figure 2 (a) Relationship between planted species richness and mean (\pm SEM) 'realized' species richness (the number of species censused within assemblages during the final spring of the experiment) in control (filled circles) and fungicide-treated (open triangles) plots across different levels of diversity. Fungicide treatment had no effect on the difference between planted and realized species richness (ANOVA, $F_{1,82} = 0.48$, $P = 0.49$). (b) Relationship between realized species richness and mean (\pm SEM) total above-ground biomass of plants in control (filled circles) and fungicide-treated (open triangles) plots. Because realized species richness diverged from planted species richness in idiosyncratic ways across individual assemblages, levels of realized species richness are not perfectly matched between treatments for all levels of realized species richness. Solid line represents significant positive relationship between diversity and productivity in control assemblages; no such positive relationship exists in fungicide-treated assemblages.

infection (McGonigle *et al.* 1990) of internal tissues by AMF vs. non-mycorrhizal (parasitic/pathogenic) fungi. These two groups were distinguished morphologically by the presence of arbuscules/vesicles/cenocytic hyphae vs. the presence of regularly septate hyphae and the absence of arbuscules and vesicles (Klironomos *et al.* 1996).

Effect of fungicide on soil N and microbial biomass

We took soil samples (using a 2.5×10 cm corer and sampling between 0 and 15 cm deep) within a subset of control and fungicide-treated plots to explore potential non-target impacts of fungicide on plant-available soil nitrogen (N) and microbial communities. Soil samples were collected in June 2009 from 27 control plots (ranging in realized species richness from 2 to 15) and 12 fungicide-treated plots spanning (ranging in realized species richness from 2 to 13). Within 2 h of collection, soil samples were sieved to 2 mm to remove roots and coarse fragments, inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) was extracted in a 2 M KCl solution (18 h), and NH_4^+ and NO_3^- in extracts were analysed colorimetrically on an autoanalyzer (Bran + Luebbe, Norderstedt, Germany). Soil microbial biomass N was determined using the chloroform fumigation-extraction method (Brookes *et al.* 1985). Fumigated (5 days) and unfumigated samples (6 g dry weight, dw) were extracted with 0.5 M K_2SO_4 for 1 h, centrifuged for 6 min at 4000 g and filtered. Filtered extracts were also analysed for NH_4^+ and NO_3^- colorimetrically using an autoanalyzer. Total N in soil extracts

was determined following digestion with potassium persulfate (D'Elia *et al.* 1977). Microbial biomass N was determined as the difference between extractable N in fumigated and unfumigated samples using a correction factor (K_f) of 0.54 (D'Elia *et al.* 1977).

Mycorrhizal responsiveness and negative soil feedbacks

To examine mycorrhizal responsiveness and the strength of negative soil feedbacks on some of the individual component species used in the experiment, we sampled soil from plots that were part of a parallel experiment (but that did not involve a fungicide treatment), where many of the same native species used in this experiment were grown in monocultures (see Maron & Marler 2007; Maron & Marler 2008 for details). Plants had grown in monoculture for 5 years prior to soil sampling, and monoculture plots were ≈ 50 m away from plots used in present experiment. We used a soil corer to remove soil (0–15 cm deep) from several randomly selected locations within each monoculture (soils sampled in summer 2008). Multiple samples from each plot were pooled and this soil was used in greenhouse experiments that were designed to shed light on two important issues. The first experiment quantified the mycorrhizal responsiveness of each native species. This allowed us to determine whether those native species that were particularly mycorrhizal responsive were negatively affected by fungicide treatment (by comparing the biomass of plants in plots sprayed or not with fungicide), as might be expected if fungicide had strong negative impacts on mycorrhizae. We inoculated 3.2 L pots containing 2.5 L of sterile sand with 0.3 L native monoculture soil that was sterilized using gamma irradiation and then had AMF spores added in or not ($n = 10$ pots per treatment and species). Spores were extracted from 10 g of field soil using a decanting-wet sieving technique (Klironomos *et al.* 1993). Spores were collected on a 45- μm sieve, surface sterilized with 10% bleach, rinsed with water and then added to the pots. After 16 weeks of growth, plants were harvested, dried and weighed. The percent change in plant biomass based on growing in sterile soil with AMF compared to growing in sterile soil without AMF represents mycorrhizal responsiveness (reported as $[\text{biomass soil with AMF}] - [\text{biomass sterile soil without AMF}] / \text{biomass sterile soil without AMF}$). The second greenhouse experiment quantified the extent to which each species in monoculture was affected by negative soil feedbacks. This allowed us to examine whether pathogen effects for mixed-species assemblages were confirmed by strong negative soil feedbacks experienced by single species when grown in monoculture soil. For this experiment, we also inoculated 3.2 L pots ($n = 10$) filled with 2.5 L γ -irradiated sand with 0.3 L of field-collected soil (from monocultures) that was either sterilized (γ -irradiated) or not (as above). We subsequently 'trained' field-collected soil by growing plants in their own monoculture soil for 16 weeks ($n = 10$ per treatment and species) and then removed these plants and planted new seeds of each native in its own soil (that had previously been sterilized or not) and grew this second round of plants for 12 weeks, after which we harvested, dried and weighed these plants. The percentage difference in biomass of plants grown in pots containing either sterilized or unsterilized field soil sampled from the native monocultures that was subsequently 'trained' (using the same plant species for 16 weeks; $n = 10$ per treatment and species) represents either positive or negative soil feedback.

Analyses

Although our manipulations varied both species and functional richness, these two variables were strongly correlated across assemblages (Pearson $r = 0.83$). As such, we focused on analysing the effects of species rather than functional richness as in full models the model fit was better for effects of realized species richness (R^2 for entire model = 0.29) compared with realized functional richness ($R^2 = 0.22$). However, given the co-variation between species and functional richness, we acknowledge that reported effects could be jointly driven by both components of diversity.

We used multi-way ANCOVA (in SYSTAT version 11, Chicago, IL, USA) to determine how block, fungicide treatment and native species richness influenced native plant productivity (log-transformed). Fungicide treatment was a fixed factor and block was a random factor. Species richness was a continuous covariate, and was log-transformed to help linearize the asymptotic relationship between native biomass and species richness. Because some species were lost from plots over the course of the experiment, we used 'realized' species richness (i.e., the actual number of species in each plot) rather than planted species richness in this analysis. We performed a one-way ANOVA to determine whether fungicide treatment influenced the magnitude of difference between planted and 'realized' species richness. Figure 2a shows how planted and realized species richness related to each other. We also used a one-way ANOVA to test for how fungicide treatment influenced species evenness (Pielou's J).

We used a two-way ANCOVA to test the effect of realized species richness and fungicide treatment on plant-available soil N and the percentage of plant roots containing parasitic fungi or AMF (arc-sin square root transformed). Interaction terms were omitted as preliminary models revealed they were not significant ($P > 0.30$).

Table 3 Mean mycorrhizal responsiveness and extent of negative soil feedback for individual species when grown in monoculture

Species	Mean mycorrhizal responsiveness (%)	Mean per cent reduction in biomass due to negative soil feedbacks (%)
<i>Festuca idaboensis</i>	-2	58
<i>Koeleria macrantha</i>	-6	46
<i>Geum triflorum</i>	32	52
<i>Penstemon procerus</i>	3	71
<i>Achillea millefolium</i>	15	77
<i>Monarda fistulosa</i>	32	41
<i>Penstemon wilcoxii</i>	-14	58
<i>Potentilla arguta</i>	23	66
<i>Gaillardia aristata</i>	-7	47
<i>Aster ericoides</i>	13	37
<i>Artemisia frigida</i>	22	49

RESULTS

The above-ground biomass of plant assemblages increased with increasing plant diversity (Fig. 2b; $F_{1,78} = 9.95$, $P = 0.002$). Soil fungicide treatment also significantly increased native plant biomass ($F_{1,78} = 9.73$, $P = 0.003$). Across all plots and diversity levels, the average production of plots increased from 128.9 g per 0.562 m² above-ground dw biomass in control assemblages to 181.4 g per 0.562 m² in fungicide-treated assemblages, an increase of 40.7%. Most notably, the slope of the diversity–productivity relationship was significantly altered by fungicide treatment (Fig. 2b; fungicide × species richness interaction; $F_{1,78} = 5.12$, $P = 0.026$). When control plots were analysed in isolation, there was a strong positive relationship between diversity and productivity ($F_{1,38} = 18.58$, $P < 0.0001$), whereas this relationship disappeared in plots treated with fungicide ($F_{1,38} = 0.40$, $P = 0.53$). The slope of the diversity–productivity relationship was greatly diminished in fungicide-treated plots because the positive effect of fungicide on native biomass was much greater in low than in high-diversity assemblages (Fig. 2b).

These results appeared to be driven by parasitic/pathogenic soil fungi. The mean percentage of root length infected by parasitic fungi was significantly lower in fungicide-treated vs. control assemblages (mean percentage of root infected control = 41% ± SEM 4.75, fungicide = 15.8% ± SEM 2.84; $F_{1,21} = 26.31$, $P < 0.0001$) and was higher in roots extracted from low- vs. high-diversity mixtures ($F_{1,21} = 4.71$, $P = 0.04$). Moreover, when individual species were grown in soil collected from their own monocultures that was subsequently ‘trained’ by the same species, all suffered substantial reductions in biomass compared to when they grew in sterilized soil (Table 3). In other words, we found evidence for strong negative soil feedbacks when individual species grew by themselves.

We found no effect of plant diversity on the extent to which roots were colonized by AMF ($F_{1,20} = 0.035$, $P = 0.85$) although AMF infection was lower in fungicide-treated vs. control plots ($F_{1,20} = 8.6$, $P = 0.008$). The effects of fungicide on AMF infection, however, were similar across the diversity spectrum (no fungicide × diversity interaction; $F_{1,20} = 0.67$, $P = 0.42$). Using data from a separate greenhouse experiment

where we quantified mycorrhizal responsiveness of species, we found that four of the five species that were most mycorrhizal responsive (*Geum triflorum*, *Monarda fistulosa*, *Potentilla arguta*, *Achillea millefolium* and *Artemisia frigida*; Table 3) were positively rather than negatively affected by fungicide in low-diversity plots, contrary to what one would expect if fungicide impacts on AMF infection strongly reduced plant performance (Table 4). Fungicide treatment did not significantly affect either plant-available soil N (mean control: 4.3 µg N g⁻¹ soil; mean fungicide: 4.1 µg N g⁻¹ soil; $F_{1,35} = 0.14$, $P = 0.71$) or microbial N (mean control: 21.7 µg g⁻¹; fungicide treatment: 21.66; $F_{1,35} = 0.003$, $P = 0.65$). Fungicide application also had no effect on species evenness (i.e., the relative abundance of each species within each assemblage; Pielou's J; $F_{1,82} = 0.002$, $P = 0.96$).

DISCUSSION

Our results illustrate that soil fungal pathogens can strongly influence the diversity–plant productivity relationship. Such influences have been generally overlooked as past work has instead focused on how competitive interactions and niche-based resource acquisition can affect plant productivity with increasing diversity. In particular, researchers have sought mechanisms to explain high plant productivity within high-diversity mixtures (Cardinale *et al.* 2007) rather than determining if processes could reduce productivity in low-diversity assemblages. Across 44 studies that manipulated plant richness, Cardinale *et al.* (2007) found that on average, diverse mixtures produced 170% more above-ground biomass than constituent species grown in monoculture. Our results illustrate a similarly strong effect of diversity. Diverse control plant mixtures (those with realized species richness of 11 species or more) produced, on average, 141% more biomass than control assemblages containing only three or fewer species. Yet, when soil fungal pathogens were suppressed by fungicide application, this impressive increase in production with increasing diversity was eliminated. This occurred because suppressing soil pathogens elevated the biomass of low-diversity assemblages, equilibrating biomass production across the diversity gradient.

Soil pathogens are increasingly being recognized as agents that can play an important role in determining characteristics of plant communities. For example, soil pathogens can impair growth and kill young plants (Van der Heijden *et al.* 2008; Packer & Clay 2000; Reinhart *et al.* 2005; Bell *et al.* 2006), with these species-specific deleterious effects accruing through time, a process known as negative soil feedback (Bever 1994). Moreover, density-dependent pathogen attack (Mills & Bever 1998; Packer & Clay 2000; Bell *et al.* 2006) can mediate competitive interactions (Alexander & Holt 1998; Kardol *et al.* 2007; Petermann *et al.* 2008), and thus critically influence the maintenance of diversity (Janzen 1970; Connell 1971; Bever 1994; Bever *et al.* 1997; Bonanomi *et al.* 2005; Bradley *et al.* 2008) or drive successional change (Van der Putten *et al.* 1993; Kardol *et al.* 2006).

Our results have several elements in common with this growing body of work. First, we found evidence that individual species suffer from strong negative soil feedbacks. In a parallel study, monocultures of many of the component species used in the present experiment declined in biomass production dramatically across the 6 years we have maintained these plots (J.L. Maron & M. Marler, unpublished data). This has been found in other experiments that have involved growing species in monoculture (Spehn *et al.* 2005). Results from a greenhouse study showed that individual species grown in their own monoculture soil suffered from substantial reductions in growth compared with plants grown in the same soil that was sterilized (Table 3). Such negative soil feedbacks may explain why monocultures declined through time. Second, our results

Table 4 Mean response of individual species to fungicide in assemblages containing five or fewer species (low-diversity assemblages) and nine or more species (high-diversity assemblages)

Species	Low-diversity assemblages			High-diversity assemblages		
	Control	Fungicide	Per cent change due to fungicide	Control	Fungicide	Per cent change due to fungicide
<i>Festuca idaboensis</i>	6.55 (10%)	11.77 (10%)	11%	7.99 (6%)	8.30 (6%)	4%
<i>Koeleria macrantha</i>	41.5 (49%)	57.71 (44%)	39%	11.78 (9%)	22.79 (16%)	93%
<i>Geum triflorum</i>	N/A	N/A	N/A	6.42 (5%)	4.10 (2%)	-36%
<i>Antennaria rosea</i>	N/A	N/A	N/A	1.61 (2%)	1.13 (0.5%)	-30%
<i>Penstemon procerus</i>	N/A	N/A	N/A	2.60 (2%)	3.48 (2%)	34%
<i>Achillea millefolium</i>	30.7 (40%)	5.7 (5%)	-81%	27.71 (12%)	17.69 (9%)	-46%
<i>Monarda fistulosa</i>	58.6 (35%)	83.87 (42%)	43%	23.57 (17%)	25.53 (15%)	8%
<i>Penstemon wilcoxii</i>	19.95 (17%)	13.15 (7%)	-34% (-10%)	24.92 (15%)	16.92 (10%)	-32% (-5%)
<i>Potentilla arguta</i>	19.8 (20%)	21.55 (11%)	9% (-9%)	11.00 (8%)	8.37 (5%)	-24% (-3%)
<i>Gaillardia aristata</i>	8.22 (8%)	16.73 (11%)	103% (3%)	8.97 (5%)	2.66 (1%)	-70% (-4%)
<i>Aster ericoides</i>	79.2 (54%)	98.9 (50%)	25% (-4%)	66.6 (43%)	27.50 (19%)	-59% (-24%)
<i>Artemisia frigida</i>	63.10 (61%)	286.50 (87%)	354% (26%)	24.29 (15%)	119.76 (52%)	393% (37%)

Numbers in parentheses indicates the average relative contribution made by each species to the total biomass of low- or high-diversity plots containing that species. *Poa sandbergii*, *Dodecatheon pulchellum* and *Lewisia rediviva* are missing from this table either because they only grew in high-diversity mixes. *Arnica sororia* is missing because it was lost from many of the plots.

suggest that soil fungal pathogens operate in a species-specific manner. That is, some plant species responded quite positively to fungicide, whereas others had more muted responses (Table 4). We caution, however, that these effects could represent either variation in the species-specific direct effects of fungicide or from indirect effects resulting from changes in the abundance of competing species. That said, if pathogens equally attacked all species then pathogen suppression might be expected to have strong effects across the entire diversity spectrum (Fig. 1b), which was not the case. Finally, because fungicide effects were stronger within low diversity assemblages that contained a few species at relatively high abundance compared to high diversity assemblages that contained more species but at lower relative abundance, it is likely that soil pathogens attacked plants in a density-dependent manner. In support of this notion, the one species that grew abundantly in both high- and low-diversity mixes, *A. frigida*, also showed evidence of being highly suppressed by soil pathogens at both low and high diversity (Table 4). Since we had a highly productive species within both low- and high-diversity mixtures it is unlikely that the positive diversity–productivity relationship in control assemblages was driven by a selection effect. If a strong selection effect was operating we might expect greater density-dependent pathogen attack at high diversity (Fig. 1c) since these plots might contain a highly productive species at higher abundance than what any given species could attain at lower diversity.

Interestingly, such a result has been found in previous work that manipulated insect herbivory across a plant diversity gradient. Mulder *et al.* (1999) found a positive plant diversity–productivity relationship that was driven by a selection effect. In their experiment, a few productive legume species were more represented in high than low-diversity plots. As insect herbivores concentrated on these species, herbivore suppression boosted productivity to a greater extent in high- vs. low-diversity assemblages (Mulder *et al.* 1999). We found the opposite results because pathogen attack was greatest in low as opposed to high-diversity plots. This suggests that plant enemies that respond in a density-dependent fashion can either enhance or diminish the diversity–productivity relationship (Fig. 1) in-part depending on how relative plant abundance is partitioned across the diversity gradient.

Could our results have been caused by unintended indirect effects of fungicides? We addressed this question in several ways. First, we examined whether fungicide treatment could release soil nutrients and increase plant biomass through this effect. In the middle of the growing season, there was no significant difference between control and fungicide-treated plots in soil inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) or on total microbial biomass N. Plant-available N also did not change across the diversity gradient. Second, observationally we saw very little evidence of insect herbivory in our plots, and certainly no difference in herbivory based on fungicide treatment. Third, to determine whether fungicide could reduce AMF root infection, we examined infection of roots harvested from control and fungicide-treated plots. Here, we did find that fungicide reduced AMF root infection, but fungicide-induced reductions in mycorrhizal root infection were similar in magnitude across the diversity gradient, contrary to what one might expect if this was an important driver of our results. More importantly, if non-target effects of fungicide on AMF were important, this would likely result in fungicide suppressing plant biomass as opposed to increasing it, as we found. Thus, in this light our results are very conservative in that control plots integrate both negative and positive impacts of soil fungi on plant growth. Finally, two of the three species that grew in low-diversity assemblages that were most mycorrhizal responsive in a separate greenhouse experiment (Table 3) were not negatively influenced by fungicide (Table 4), as one might expect of fungicide-induced suppression of mycorrhizal infection negatively effected plant performance. Although we did not examine fungicide effects on soil food web structure, saprophytic fungi or net N mineralization rates, for these non-target effects to drive our results they would have to be extremely large, positive rather than negative for plant growth, and operate differently within low- vs. high-diversity assemblages. In theory this could occur if fungicide treatment resulted in: (1) greater amounts of nutrients being made available within low- vs. high-diversity assemblages or (2) a similar but high increase in nutrient availability across the diversity gradient but with more uptake in low- vs. high-diversity plots. Either of these effects, however, would have to be large enough in plots with five species or less to cause the 98% increase in plant biomass that we found in fungicide-treated assemblages compared with controls and yet not be reflected in measures of free NO_3^- and NH_4^+ in the soil. A more parsimonious interpretation of our results is that pathogens accumulated to a greater extent, and thus had greater impacts on plant biomass, in low than high-diversity plots. This interpretation is supported by the fact that fungicide both reduced the extent of root infection by parasitic fungi, and that these effects were greatest in low- vs. high-diversity mixtures. It is further supported by the fact that not all the species that grew in lower diversity plots (those containing five or fewer species) responded similarly to fungicide application (Table 4). As we could not tease apart effects of species and functional richness on productivity, determining which component of diversity more strongly affects productivity and pathogen attack awaits further study.

Our results, and those of a similar study to ours showing important impacts of soil microbes on the diversity–productivity relationship (Schnitzer *et al.* in press), have implications for how small-scale diversity–productivity relationships might be interpreted. In the past, there has been almost exclusive focus on complementarity

and the selection effect as single mechanisms that can produce the positive diversity–productivity relationship, and how the strength of these mechanisms might change through time (Cardinale *et al.* 2006, 2007; Fornara & Tilman 2009). Results from our work and Schnitzer *et al.* (in press) suggest that soil pathogens may be a neglected but potent force that can also drive a positive diversity–productivity relationship. We speculate that patterns of soil pathogen attack, and therefore the impacts pathogens have on plant community productivity, could also very much depend on whether a selection effect or complementarity are also operating (see Fig. 1). If true, niche-based/competitive processes and soil pathogen effects are not mutually exclusive mechanisms but rather may act in concert. At the same time, since suppression of pathogens eliminated the positive diversity–productivity relationship, in our system any resource-based mechanism that does operate in the presence of pathogens is clearly not strong enough to drive a positive diversity–productivity relationship when those pathogens are suppressed. Beyond diversity–productivity relationships, our findings suggest that soil pathogens could strongly maintain diversity by reducing the performance of any species that becomes strongly dominant in mixed-species assemblages (Bever *et al.* 1997). Thus, negative density-dependent effects of soil pathogens may both suppress the productivity of low-diversity assemblages, while helping to maintain the diversity of more species-rich assemblages.

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