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REPORT

Trophic control of grassland production and biomass by pathogens

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Abstract

Current theories of trophic regulation of ecosystem net primary production and plant biomass incorporate herbivores, but not plant pathogens. Obstacles to the incorporation of pathogens include a lack of data on pathogen effects on primary production, especially outside agricultural and forest ecosystems, and an apparent inability to quantify pathogen biomass. Here, I report the results of an experiment factorially excluding foliar fungal pathogens and insect herbivores from an intact grassland ecosystem. At peak in control plots, 8.9% of community leaf area was infected by pathogens. Disease reduction treatment dramatically increased root production and biomass by increasing leaf longevity and photosynthetic capacity. In contrast, herbivory reduction had no detectable effects at the ecosystem or leaf scale. Additionally, biomass of foliar fungal pathogens in the ecosystem was comparable with that of insect herbivores. These results identify pathogens as potential regulators of ecosystem processes and promote the incorporation of pathogens into trophic theory.

Keywords

Community structure, disease, ecosystem processes, host–pathogen, net primary production, parasites, productivity, top-down, trophic regulation, trophic structure.

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INTRODUCTION

Like insect herbivores (Brown & Gange 1989; Oerke *et al.* 1994; Root 1996; Carson & Root 2000; Schowalter 2000; Hunter 2001), plant pathogens decrease global crop production by roughly 15% (Oerke *et al.* 1994), can regulate forest ecosystem processes (Castello *et al.* 1995; Hansen & Goheen 2000), and can control the structure and dynamics of plant communities and populations (Burdon 1987; Dobson & Crawley 1994; Castello *et al.* 1995; Jarosz & Davelos 1995; Peters & Shaw 1996; Hansen & Goheen 2000; Packer & Clay 2000; Klironomos 2002; Strengbom *et al.* 2002). While an extensive body of theory describes host–pathogen population dynamics (e.g. Grenfell & Dobson 1995), pathogens and parasites have not been included in the theories of trophic structure central to ecosystem ecology (Polis & Strong 1996; Polis 1999; Chapin *et al.* 2002).

Two major obstacles to the inclusion of pathogens in trophic theory result from lack of empirical data. First, pathogen biomass has almost never been quantified for any ecosystem (but see Smith *et al.* 1992), allowing the common assumption that pathogen biomass is negligible to persist (Polis & Strong 1996; Polis 1999). This assumption renders pathogens incompatible with trophic theory because ecosystem structure and processes are quantified in terms of masses of pools (e.g. plant biomass) and in fluxes between pools [e.g. net primary production (NPP)], based on the principle of mass-balance (Polis & Strong 1996; Polis 1999; Chapin *et al.* 2002). Second, perhaps because most pathogens infect only a subset of plant species in an ecosystem (Burdon 1987), the effects of pathogens on ecosystem processes and structure aggregated across all species in the ecosystem have been little studied. For example, the effects of plant pathogens on NPP and plant biomass have only been quantified for agricultural and forest ecosystems (e.g. Wright & Gaunt 1992; Castello *et al.* 1995; Hansen & Goheen 2000). Even in these ecosystems, data on belowground NPP and biomass are rare, as are data on NPP and biomass per se, rather than yield to humans.

Foliar fungal pathogens are long-term parasitic sinks for leaf nutrients and photosynthate (Burdon 1987; Bastiaans 1991; Farrar 1992; Jarosz & Davelos 1995; Lopes & Berger 2001); some also release phytotoxins (Bastiaans 1991; Lopes & Berger 2001). As a result, they commonly decrease net photosynthetic capacity and leaf longevity (Burdon 1987;

Bastiaans 1991; Jarosz & Davelos 1995; Lopes & Berger 2001). Plant allocation theory predicts that such decreases in photosynthesis and leaf longevity will decrease root biomass more than shoot biomass (Bloom *et al.* 1985; Walters 1985; Farrar 1992), which has been reported for individual plants (Walters 1985; Burdon 1987). The effects of foliar fungal pathogens on photosynthesis commonly follow the relationship

$$\frac{P_x}{P_0} = (1 - x)^{\beta} \quad (1)$$

where P_x is the photosynthetic capacity of a leaf with x proportion of its area covered by the pathogen, P_0 is the mean photosynthetic capacity of uninfected control leafs, and β, called the ratio of virtual to visual lesion size and representing the degree to which infection suppresses photosynthesis, is estimated by nonlinear regression (Bastiaans 1991; Lopes & Berger 2001). For crop pathogens, β can be as high as 12 (Lopes & Berger 2001), indicating that even at low infection intensity, such as in non-agricultural ecosystems (Burdon 1987; Jarosz & Davelos 1995; Peters & Shaw 1996; Mitchell *et al.* 2002; Schnitzer *et al.* 2002; Mitchell *et al.* in press), foliar fungal pathogens can have strong negative effects on their hosts. Together, theory and empirical data suggest the untested hypothesis that foliar fungal pathogens can limit below-ground NPP and root biomass in non-agricultural ecosystems by decreasing photosynthetic capacity and leaf longevity.

Here I report the results of a 3-year field experiment testing this hypothesis in intact grassland at Cedar Creek Natural History Area, Bethel, MN, USA. Foliar fungi and insects were factorially excluded using pesticides. The insect exclusion treatment was included to compare the effects of pathogens and those of a group, insect herbivores, known to be potentially important regulators of ecosystem processes. Data presented are from 1999 unless otherwise noted; more limited data from previous years on pathogen load, herbivory, above-ground production, soil respiration and leaf longevity support these results. Additionally, a greenhouse experiment was conducted to quantify any effects of the experimental pesticide treatments on plant growth resulting from mechanisms other than disease and herbivory reduction (Brown & Gange 1989; Paul *et al.* 1989; Peters & Shaw 1996; Carson & Root 2000).

MATERIALS AND METHODS

Field experiment

The field experiment was conducted at Cedar Creek Natural History Area, Bethel, MN, USA. The experimental field was last cultivated in 1943 and has since undergone natural succession. Today, it is dominated by a native perennial grass, *Andropogon gerardi*, although the non-native perennial grass *Poa pratensis* is the second most abundant species. Thirty-two 6.25 m^2 plots, separated by 1 m aisles, were established in July 1996. From then through 1999, non-systemic, broad-spectrum fungicide (mancozeb) and insecticide (esfenvalerate) were applied as sprays approximately every 7–10 days through the growing season, generally May to September. The experiment was a completely randomized factorial design with eight replicates for each of the four treatment combinations.

Soil respiration was measured in July and September 1999 using a Li-Cor LI-6200 gas exchange system and Li-Cor 6400-09 soil respiration chamber (Li-Cor Inc., Lincoln, NE, USA), then averaged over time. Ecosystem root biomass was quantified by washing, drying and weighing three 6.35 cm diameter, 25 cm deep cores per plot on 26 June 1999. Ecosystem root production was quantified as ingrowth by refilling the cored holes with sieved soil, then extracting 5.1 cm diameter, 20 cm deep cores on 14 September 1999. Above-ground plant biomass was clipped from two 1.5 m × 0.1 m strips per plot in mid-August 1999, its seasonal peak, then sorted to species, dried and weighed. Above-ground and below-ground plant biomass were summed to calculate estimates of total plant biomass and root fraction. Because above-ground biomass turns over annually, its annual peak provides an estimate of annual above-ground NPP. We did not attempt to calculate total NPP because above-ground and below-ground NPP were not measured over the same time interval. Before starting treatments in 1996, and each July thereafter, percentage cover of all plant species (a measure of their relative abundances) was estimated in two permanent 1.0 m × 0.5 m quadrats per plot using cardboard cutouts of known area as visual guides. Total percent cover in each quadrat, including cover of bare ground and exposed litter, summed to 100%.

In contrast to the measures above, which included all plant species in all plots, leaf-level sampling was conducted only in half of the plots (randomly chosen) of each factorial treatment, and focused on the four most abundant species because they comprised 95% of the total above-ground biomass sampled by clipping in 1999. Each species was naturally infected by a pathogenic asexual ascomycete fungus causing a leaf spot disease: *A. gerardi* by *Phyllosticta* sp., *P. pratensis* by *Ascochyta* sp. (tentative), *Schizachyrium scoparium* by *Colletotrichum* sp., and *Carex foenea* by *Septoria* sp. Additionally, *Andropogon* was infected by the rust fungus *Puccinia andropogonis*. Voucher specimens were deposited in the University of Minnesota Herbarium. From June to October 1999, leaf longevity was quantified by tracking the growth and senescence of each leaf on two to three tillers of each species in each plot sampled every 2 weeks (Craine &

Reich 2001). Each time these leaves were censused, the percentages of leaf area infected by fungal pathogens and consumed by chewing and mining insects were also estimated by comparison with images of leaves of known percentage area damaged (Campbell & Madden 1990). This allowed calculation of the areas under the disease and herbivory progress curves (AUDPC and AUHPC), which quantify damage integrated over time (Campbell & Madden 1990). These were integrated over the entire growing season except when testing their correlation with root production (Fig. 4), when they were integrated over the interval of root production only. 'Community-wide' values for leaf longevity and areas under the disease and herbivory progress curves were calculated as biomass-weighted averages of the four species sampled (Mitchell *et al.* 2002) based on the assumption that they represented the entire community. Maximum net photosynthetic rate per area of the dominant species, *A. gerardi* (58% of total above-ground biomass), was quantified on 27 August 1999 using a CIRAS-1 portable infrared gas exchange system (PP Systems, Hitchin, UK). For each tiller tracked for leaf longevity, photosynthesis was measured for the youngest section of the oldest leaf with sufficient green length (9 cm) to fill the narrow leaf cuvette.

Pesticide selection

Esfenvalerate contains the same components as fenvalerate, an insecticide used in several ecological experiments (Root 1996; Carson & Root 2000), but has a higher concentration of the one insecticidal isomer. Therefore, it is applied at a lower rate than fenvalerate, should have lesser non-target effects, and has replaced fenvalerate for use in the USA. A recent review concluded that fenvalerate is an ideal choice for herbivory reduction experiments because it is not phytotoxic, has minimal influence on pollinators, has no effects on soil microbes or nutrients and degrades quickly after binding to surface soil and litter (Carson & Root 2000); this should be equally true for esfenvalerate.

Mancozeb has been used worldwide for over 40 years. It does not have any direct effects on photosynthesis (Stamps & Chase 1987; Lorenz 1989) leaf longevity (Stamps & Chase 1987; Lorenz 1989), shoot growth (Lorenz 1989; Kope & Trotter 1998), or root growth (Lorenz 1989; Kope & Trotter 1998). On vegetation, mancozeb's half life is 10 days (Kumar & Agarwal 1992). In soil, it remains in the top 2.5 cm (Calumpang *et al.* 1993) and breaks down with a half life of 4 days or less (Doneche *et al.* 1983; Calumpang *et al.* 1993). Mancozeb is broken down primarily through abiotic processes such as photooxidation (Doneche *et al.* 1983; Rajagopal *et al.* 1984), and its breakdown products are undetectable after 2–6 weeks in soil (Calumpang *et al.* 1993). Mancozeb treatment added *c.* 0.50 g N m^{-2} $year^{-1}$. Effects of mancozeb on arbuscular mycorrhizae are variable. An extensive review concluded for dithiocarbamates, including mancozeb, that 'recommended rates of field application frequently seem not to affect mycorrhizae' (Trappe *et al.* 1984). A recent study examining the effects of foliar mancozeb spray on mycorrhizae found that it had no effect on mycorrhizal colonization of leek, but reduced that of wheat by 12% (Plenchette & Perrin 1992).

Greenhouse experiment

To test for non-target effects of the pesticide sprays, the three most abundant plant species in the field experiment (*Andropogon*, *Poa* and *Schizachyrium*) were grown in a greenhouse in which effort was made to avoid introducing pathogens and herbivores from the field. Seed was obtained from Prairie Restoration Inc. (Princeton, MN, USA); *Andropogon* and *Schizachyrium* were local ecotypes. Plants were grown in unsterilized soil from the study site. Therefore, any effects observed in the field experiment resulting from direct effects of the pesticides on the plants, soil biota, or soil chemistry should have been reproduced in the greenhouse. Pots (1.6 l volume) were watered daily to field capacity and temperature was maintained at *c.* 25°C. Natural daylight was supplemented with 450 μmol $m^{-2}s^{-1}$ of photosynthetically active radiation via halogen lamps from 06:00 to 20:00 hours to simulate a summer growing season. Each species was grown separately to facilitate quantification of root biomass by species. Thirteen individuals of each species were transplanted from germination flats to each pot. Starting 10 weeks after germination, pesticide treatments were applied at the same rate as in the field experiment for 4 months, approximating one field season of pesticide applications. Each factorial combination of fungicide and insecticide was replicated eight times for *Andropogon* and *Schizachyrium*, and five times for *Poa* because 12 pots of plants infected with powdery mildew were destroyed a month before beginning pesticide treatments. Pathogen infection throughout the rest of the experiment was at almost undetectably low levels (see Results), so the experiment tested the effects of the pesticides in a nearly disease- and herbivory-free environment. Approximately every 6 weeks after beginning treatments, percentages of leaf area infected and eaten were determined for 10 leaves in each pot as in the field experiment. Additionally, the percentage of leaf area remaining green was visually estimated and integrated over time as a proxy for leaf longevity. At the end, shoots and roots were separated, washed, dried and weighed similarly as in the field.

Analysis

Total sample size for analyses including leaf-level variables was half that (16) of other analyses (32) because leaf-level

variables were only sampled in half the plots of each factorial treatment. Analyses, except nonlinear regression, were performed in SYSTAT 9 and based on unweighted ordinary least squares and type III sums of squares. Nonlinear regression was performed in CurveExpert 1.37 using the Levenberg-Marquardt algorithm. Plots were treated as the experimental units. Unless otherwise stated, all analyses were ANOVAs with one dependent variable and fungicide treatment, insecticide treatment and their interaction as independent variables. For the three major sets of analyses (Figs 1–3), protected ANOVAs were used: a MANOVA was used to test for overall treatment effects on a set of potentially correlated response variables, then univariate ANOVAs used to examine effects on individual response variables. To test for treatment effects on community species richness (of all species encountered in the percentage cover quadrats), a repeated measures ANCOVA was performed with species richness in 1997, 1998 and 1999 as the dependent variables and 1996 species richness as a covariate. To test for treatment effects on plant community structure, a similar repeated measures MANCOVA was performed with the 1996–99 percentage covers of the four most abundant species as the covariate and dependent variables. In MANOVAs and MANCOVAs, results shown are for Pillai's trace; Wilks' lambda and Hotelling–Lawley's trace always gave nearly identical results. Data from the greenhouse experiment were analysed using ANOVAs with species, fungicide treatment, insecticide treatment and all interactions as independent variables.

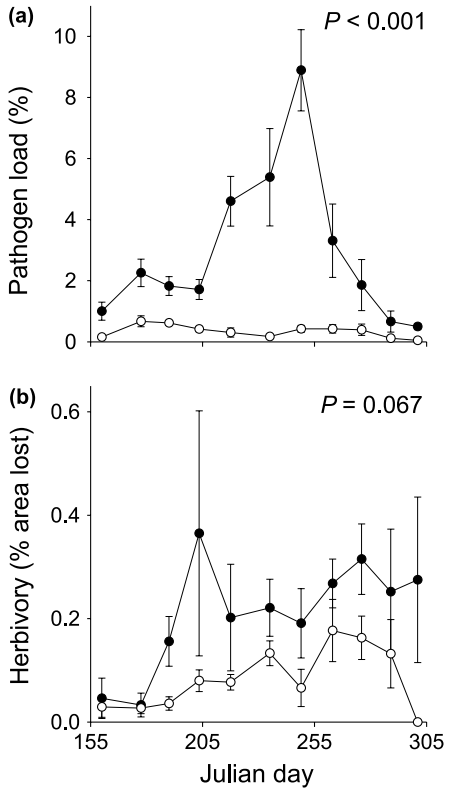

Figure 1 Effect of disease reduction treatment on disease and of herbivory reduction treatment on herbivory. (a) Disease progress curves for plots treated with (open circles) and without fungicide (filled circles). Pathogen load is the percentage of leaf area infected across the plant community. (b) Herbivory progress curves for plots treated with (open circles) and without insecticide (filled circles). *P*-values reported are for treatment effects on area under the disease or herbivory progress curve (AUDPC, AUHPC). Data shown are mean ± SEM ($n = 8$ per point). MANOVA results with AUDPC and AUHPC as dependent variables: intercept: $F_{2,11} = 60.3$; $P < 0.001$; disease reduction: $F_{2,11} = 29.1$; $P < 0.001$; herbivory reduction: $F_{2,11} = 1.64$; $P = 0.238$; interaction: $F_{2,11} = 0.984$; $P = 0.404$.

RESULTS

Field experiment

Disease reduction decreased AUDPC, the percentage community leaf area infected integrated over the entire growing season, by 81% (Fig. 1a; $F_{1,12} = 58.630$; $P < 0.001$). Area under the herbivory progress curve was 58% lower under herbivory reduction than in controls, but this difference was not significant, perhaps because <1% of leaf area was consumed even in controls (Fig. 1b; $F_{1,12} = 4.060$; $P = 0.067$). Neither herbivory reduction nor its interaction with disease reduction influenced any other response variable ($P > 0.1$), so only statistics for disease reduction are presented further.

At the ecosystem level, disease reduction increased root production by 34% relative to controls (Fig. 2a; $F_{1,28} = 6.697$; $P = 0.015$). This increased rate of production allowed 47% more root biomass to accumulate under disease reduction than in controls within 3 years (Fig. 2b; $F_{1,28} = 17.166$; $P < 0.001$). In turn, soil respiration was 18% greater under disease reduction than in controls (Fig. 2c; $F_{1,28} = 10.3$; $P = 0.003$). Above-ground production, estimated by peak above-ground biomass, was not significantly greater under disease reduction (Fig. 2d; $F_{1,28} = 0.417$; $P = 0.524$). Thus, disease reduction increased total plant biomass by 31% ($F_{1,28} = 12.696$; $P = 0.001$) and increased the root fraction of biomass from 0.60 to 0.68 ($F_{1,28} = 9.455$; $P = 0.005$). Disease reduction did not alter the relative abundances of the four most abundant species ($F_{1,24} = 2.191$; $P = 0.152$), or plant species richness ($F_{1,27} = 1.090$; $P = 0.306$).

At the leaf level, disease reduction increased by 32% the photosynthetic capacity of the species comprising 58% of total above-ground biomass, *Andropogon* (Fig. 3a; $F_{1,12} = 5.163$; $P = 0.042$). Controlling for leaf age and/or subtracting leaf area covered by the pathogen did not

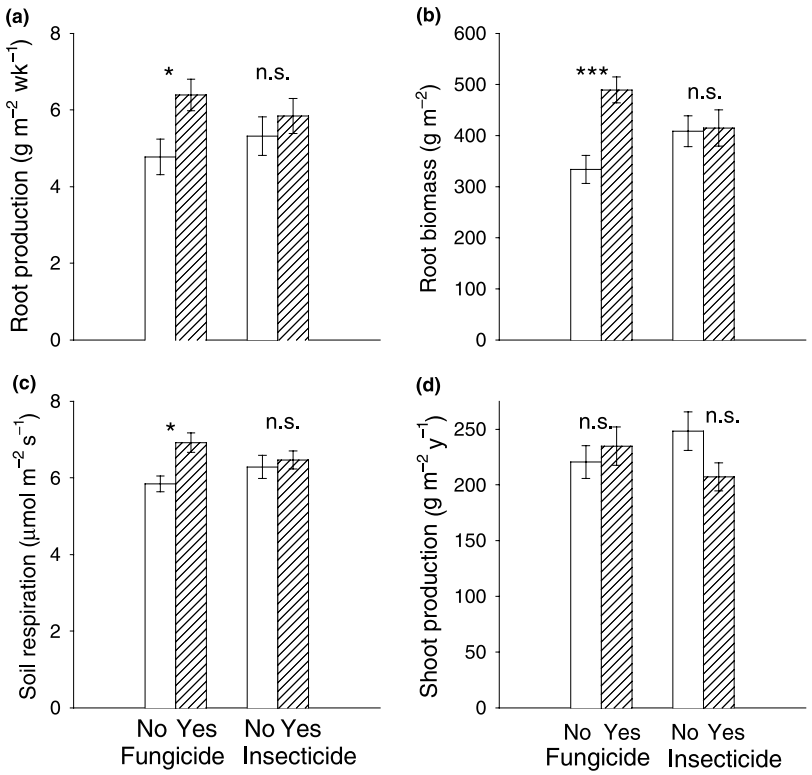

Figure 2 Effects of disease and herbivory reduction on ecosystem processes. (a) Below-ground production. (b) Root standing stock. (c) Soil carbon flux. (d) Above-ground production. Data shown are mean ± SEM ($n = 16$ per bar). *$P \leq 0.05$; ***$P \leq 0.001$; ns $P > 0.1$. MANOVA results with root production, root biomass, soil respiration and shoot production as dependent variables: intercept: $F_{4,25} = 442$, $P < 0.001$; disease reduction: $F_{4,25} = 5.90$, $P = 0.002$; herbivory reduction: $F_{4,25} = 1.15$, $P = 0.356$; interaction: $F_{4,25} = 0.949$, $P = 0.452$.

alter this effect. Performing a nonlinear regression following eqn 1 showed that β, the exponent describing the degree to which infection suppresses photosynthesis, was 3.6 ± 0.23 (mean ± SE). Disease reduction also increased community leaf longevity by 23% (Fig. 3b; $F_{1,12} = 5.075$; $P = 0.044$).

Root production was negatively correlated with AUDPC (Fig. 4; $r^2 = 0.576$; $t_{14} = -4.365$; $P = 0.001$). When disease reduction treatment was added to this model, the effect of AUDPC remained almost significant ($F_{1,13} = 3.281$; $P = 0.093$) while the effect of disease reduction treatment was not significant ($F_{1,13} = 0.007$; $P = 0.936$). Thus, controlling for AUDPC was sufficient to explain the effect of disease reduction treatment on root production, suggesting that this treatment effect resulted from decreased foliar disease. Further, root production was positively correlated with root biomass (measured immediately before production) in a simple regression ($r^2 = 0.441$; $t_{14} = 3.326$; $P = 0.005$), but not after controlling for AUDPC in a multiple regression ($r^2 = 0.581$; biomass: $t_{13} = 0.360$; $P = 0.725$; AUDPC: $t_{13} = -2.078$; $P = 0.058$), emphasizing that foliar disease was a major control on root production.

Greenhouse experiment

For all three species, peak percentage leaf area infected in any treatment in the greenhouse was less than half that observed under fungicide treatment in the field and was not influenced by either pesticide treatment or any interaction ($P > 0.15$). No herbivory was detected in the greenhouse. Neither pesticide treatment nor any interaction had any effect on percentage leaf area remaining green over time ($P > 0.39$). Shoot biomass was not influenced by either pesticide treatment or any interaction ($P > 0.18$). Root biomass was not influenced by either pesticide treatment or their interaction ($P > 0.88$), but fungicide treatment interacted with species identity ($P = 0.045$). However, post-hoc contrasts performed to understand the nature of this interaction revealed that no species' root biomass was significantly influenced by fungicide treatment. The species closest to being significantly affected was *Poa*, for which root biomass tended to be less under fungicide treatment ($P = 0.064$). Thus, in the near absence of foliar pathogens, fungicide application produced no effects similar to those observed in the field in the presence of pathogens, suggesting that the effects in the field resulted from foliar disease reduction, not unintended mechanisms.

Estimation of pathogen biomass

Because most fungal infections were restricted to leaves, leaf biomass was calculated. Mean peak total above-ground plant biomass in non-fungicide plots of the field experiment was 220 g m^{-2}. Eighty-four per cent of this biomass was leaves

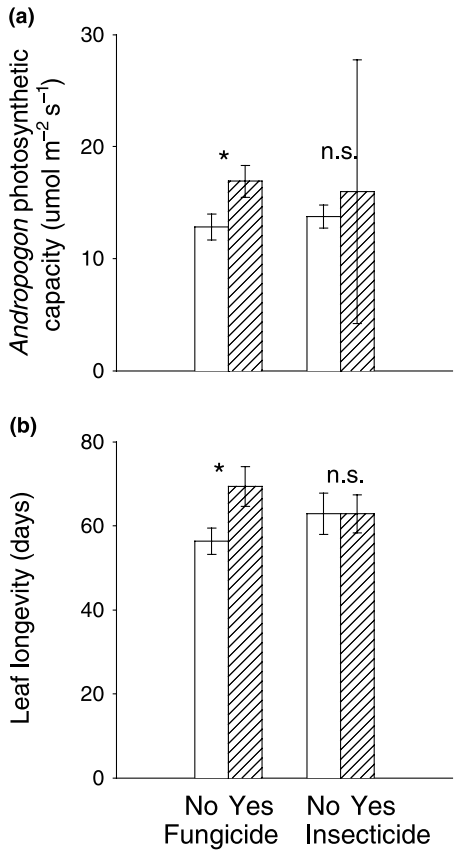

Figure 3 Effects of disease and herbivory reduction on leaf functioning. (a) Maximum net photosynthesis of the dominant species, *Andropogon gerardi*. (b) Leaf life-span across the plant community. Data shown are mean ± SEM ($n = 8$ per bar). *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ns $P > 0.05$. MANOVA results with *Andropogon* photosynthesis and community leaf longevity as dependent variables: intercept: $F_{2,11} = 357$; $P < 0.001$; disease reduction: $F_{2,11} = 4.90$; $P = 0.030$; herbivory reduction: $F_{2,11} = 0.706$; $P = 0.515$; interaction: $F_{2,11} = 1.16$; $P = 0.350$.

based on the biomass-weighted average of *Andropogon*, *Poa*, and *Schizachyrium* allocation of above-ground biomass to leaves in monocultures at the study site (Craine *et al.* 2002). These species were the three most abundant in the experiment, comprising 89% of above-ground biomass. Multiplying this proportional allocation to leaves by the observed above-ground biomass yields a total leaf biomass of 184.8 g m^{-2}.

To convert from leaf biomass to fungal biomass, published data relating infection intensity to ergosterol mass and ergosterol mass to fungal biomass were used. Ergosterol is a sterol unique to fungi. Linear interpolation of published data relating percentage wheat leaf area infected by *Septoria nodorum* to leaf ergosterol content (Griffiths *et al.* 1985) indicates that an average leaf in the experiment with 8.9% of its area infected at peak contained 15.12 μg ergosterol g^{-1} leaf. *Septoria nodorum* is an asexual ascomycete fungus causing pathogenic leaf spots. All fungi found in the experiment except *Puccinia* share these traits, making *Septoria* both ecologically and phylogenetically representative of them. Four other plant pathogenic ascomycete fungi (*Alternaria alternata*, *Fusarium solani*, *Mycosphaerella* sp. and *Pleospora spartinae*) contain an average of 3.2 mg ergosterol g^{-1} fungus (Djajakirana *et al.* 1996). Combining these two ergosterol conversion factors yields 0.0047 g fungi g^{-1} leaf. This quantity implies that 0.47% of the measured mass of an average leaf with 8.9% of its surface area visibly infected was actually fungal tissue. Finally, multiplying 0.0047 g fungi g^{-1} leaf by 184.8 g leaf m^{-2} yields 0.87 g fungus m^{-2} peak pathogen biomass.

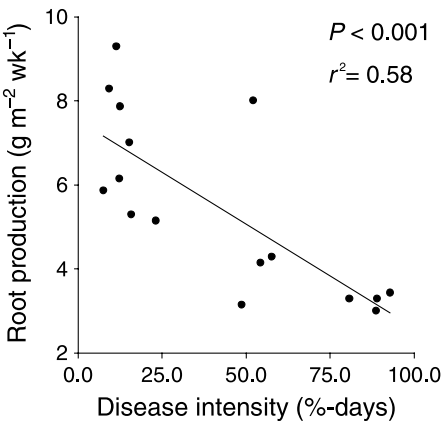

Figure 4 Correlation between root production and area under the disease progress curve (pathogen load integrated over the period of root production).

DISCUSSION

In the field experiment, reducing foliar fungal disease greatly increased below-ground plant carbon allocation, as predicted. The large increases in below-ground biomass and production occurred in an ecosystem already long renowned for high root biomass (Weaver 1958; Wilson & Tilman 1991; Craine *et al.* 2002). The observed increase in soil respiration matches that expected from the increased root biomass alone, assuming that root respiration accounted for 39% of total soil respiration as observed in a similar field at the study site (Craine *et al.* 1999). This implies that pathogens were decreasing plant carbon allocation to root biomass much more than to pools with shorter residence times such as root respiration, exudation and turnover. Therefore, on long time-scales, foliar fungal pathogens might help limit ecosystem carbon storage.

Disease reduction increased photosynthesis and leaf longevity, the major mechanisms by which foliar fungi

reduce crop yields (Burdon 1987; Bastiaans 1991; Jarosz & Davelos 1995; Lopes & Berger 2001). The observed inhibition of net photosynthesis by fungi, while large, would be typical for crop pathogens. For *Phyllosticta* on *Andropogon*, the parameter β describing the strength of this inhibition was 3.6, well within the range for crop pathogens, $0 < \beta < 13$ (Bastiaans 1991; Lopes & Berger 2001). The combination of increased photosynthesis and leaf longevity under disease reduction would be expected to have increased ecosystem annual net maximum photosynthesis by 34–62% or more, depending on the degree to which the observed effect on August *Andropogon* photosynthesis held for the rest of the community and growing season. Thus, enhanced photosynthesis and leaf longevity provide a likely mechanism for the increased below-ground carbon fluxes resulting from disease reduction.

The lack of a significant effect of disease reduction treatment on root production after controlling for AUDPC suggests that mechanisms for the effects of the treatment other than those resulting from reduced foliar disease were unimportant. Direct evidence also indicates that other possible mechanisms are unlikely to explain the observed results. The greenhouse experiment revealed no direct effects of the pesticides on plant growth. Disease reduction did not alter plant community structure. The rate of N addition from fungicide was one-twentieth of what would be required to explain the observed proportional increase in root biomass (Wilson & Tilman 1991). Several lines of evidence suggest that effects of fungicide on soil biota are an unlikely mechanism. First, plants in the greenhouse experiment were grown in unsterilized field soil, but their production was unaffected by fungicide treatment. Second, fungicide treatment in the field experiment did not decrease percentage root colonization by arbuscular mycorrhizal or non-mycorrhizal (including pathogenic) fungi (P.G. Avis and C.E. Mitchell, unpublished work). Third, mancozeb has no effect on total numbers of soil nematodes, ciliates, testaceans and rotifers (Petz & Foissner 1989). Fourth, soil drenches of mancozeb can decrease microbial respiration, net nitrification and sometimes net N mineralization (Saive *et al.* 1975; Doneche *et al.* 1983). Thus, if the foliar spray used here affected the soil biota similarly as do soil drenches, these negative effects would make the observed effects of disease reduction on NPP and soil respiration conservative.

In this study, pathogens' effects on ecosystem processes exceeded those of plants' better-studied natural enemies, insect herbivores. However, herbivores consumed <1% of leaf area, presumably because of the plants' low foliar nitrogen concentration (Mattson 1980; Craine *et al.* in press). Insects' effects should be greater in ecosystems where more palatable plants, such as many forbs (Root 1996; Carson & Root 2000), are more abundant.

Ecosystem ecology is based on the principle of mass-balance (Polis & Strong 1996; Polis 1999; Chapin *et al.* 2002), but foliar fungal pathogen biomass is inseparable from host biomass. Pathogen biomass was therefore estimated, using field and published data, to be 0.87 g m^{-2} in plots not receiving fungicide. This is the first estimate of the biomass of foliar fungal pathogens for any ecosystem. More direct methods of quantifying pathogen biomass should be developed because this estimate was based on several uncertain assumptions. However, even if this estimate were an order of magnitude too high, pathogen biomass would be comparable with insect herbivore biomass, which was 0.1–0.5 g m^{-2} in a similar field at the study site (Ritchie 2000). This finding contrasts with the common assumption that pathogen biomass is a negligible component of ecosystem trophic structure (Polis & Strong 1996; Polis 1999).

While pathogens are well integrated into population and community ecology (Burdon 1987; Dobson & Crawley 1994; Castello *et al.* 1995; Jarosz & Davelos 1995; Peters & Shaw 1996; Hansen & Goheen 2000; Packer & Clay 2000; Klironomos 2002; Strengbom *et al.* 2002), they have generally been viewed as being outside of the central framework of ecosystem ecology (Polis & Strong 1996; Polis 1999; Chapin *et al.* 2002). By highlighting the potential for pathogens comprising small but measurable amounts of biomass to control major ecosystem pools and fluxes, these results help begin to integrate pathogens into this framework.

Foliar fungi reduced plant carbon gain and thus below-ground allocation at current ambient pathogen loads, with a maximum of 8.9% of community leaf area infected. However, recent studies indicate that components of environmental change such as decreased plant species diversity (Mitchell *et al.* 2002; Mitchell *et al.* in press), nitrogen deposition (Strengbom *et al.* 2002; Mitchell *et al.* in press) and climate warming (Harvell *et al.* 2002) can increase foliar fungal pathogen loads. In the light of these studies, results here suggest that increased pathogen loads may limit the ability of ecosystems to sequester carbon below-ground in response to environmental change.

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