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Warming and fertilization alter the dilution effect of host diversity on disease severity

Xiang Liu,[1] Shengman Lyu,[1] Shurong Zhou,[1,3] and Corey J. A. Bradshaw[2]

[1]*Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, School of Life Sciences, Fudan University, 2005 Songhu Road, Shanghai 200438 China*
[2]*School of Biological Sciences, University of Adelaide, South Australia 5005 Australia*

Abstract. An essential ecosystem service is the dilution effect of biodiversity on disease severity, yet we do not fully understand how this relationship might change with continued climate warming and ecosystem degradation. We designed removal experiments in natural assemblages of Tibetan alpine meadow vegetation by manipulating plot-level plant diversity to investigate the relationship between different plant biodiversity indices and foliar fungal pathogen infection, and how artificial fertilization and warming affect this relationship. Although pathogen group diversity increased with host species richness, disease severity decreased as host diversity rose (dilution effect). The dilution effect of phylogenetic diversity on disease held across different levels of host species richness (and equal abundances), meaning that the effect arises mainly in association with enhanced diversity itself rather than from shifting abundances. However, the dilution effect was weakened by fertilization. Among indices, phylogenetic diversity was the most parsimonious predictor of infection severity. Experimental warming and fertilization shifted species richness to the most supported predictor. Compared to planting experiments where artificial communities are constructed from scratch, our removal experiment in natural communities more realistically demonstrate that increasing perturbation adjusts natural community resistance to disease severity.

Key words: alpine meadow; biodiversity; community phylogeny; diversity–disease relationship; foliar fungal disease; species evenness; species richness; Tibetan Plateau.

Introduction

Quantifying the complex relationships between biological diversity, ecosystem functions and the services they provide are some of the central pursuits of community ecology (Tilman et al. 2014), and their importance is increasing as species extinctions accelerate during the Anthropocene (Pimm et al. 2014). One biodiversity—ecosystem service relationship that is receiving increasing attention is how biodiversity affects the severity of infectious diseases in a variety of species, including plants (Keesing et al. 2010, Cardinale et al. 2012, Johnson et al. 2013).

There are two opposing hypotheses relevant to the complex relationship between host species diversity and infectious disease: (1) a “dilution” effect arises when disease risk and prevalence decrease with increasing host species diversity, while (2) the “amplification” hypothesis predicts an increase in disease as host species diversity rises (Keesing et al. 2006, Ostfeld and Keesing 2012). A dilution effect can arise by several general mechanisms such as encounter reduction, transmission interference, and susceptible-host regulation in diverse communities (Keesing et al. 2006). On the contrary, diverse host communities can potentially harbor greater diversity and abundance of pathogens, thus leading to accelerated incidences of the transmission and outbreak of diseases, resulting in an amplification effect (Keesing et al. 2010, Wood et al. 2014). Despite studies in natural and artificial ecosystems overwhelmingly supporting the dominance of dilution instead of amplification (e.g., Haas et al. 2011, Johnson et al. 2013, 2015, Miller and Huppert 2013, Civitello et al. 2015), controversy persists regarding the mechanisms involved.

Although several studies have shown that community composition and species identity both play important roles in explaining the emergence and transmission of diseases (Hantsch et al. 2013, 2014, Lacroix et al. 2014, Rottstock et al. 2014), most previous studies investigating the myriad effects of diversity on disease have relied on the simple metric of species richness as a surrogate measure of host diversity (e.g., Johnson et al. 2008, Lacroix et al. 2014, Rottstock et al. 2014). In fact, biodiversity can be quantified in many different ways, such as simple species richness, evenness, functional diversity, and phylogenetic diversity, with different metrics demonstrating variable power to predict ecosystem functioning (Faith 1992, Srivastava et al. 2012, Cadotte et al. 2013, Liu et al. 2015). Using functional diversity as a metric to predict ecosystem functions such

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[3]E-mail: zhshrong@fudan.edu.cn

as disease risk is largely limited to the selection of appropriate traits related to disease emergence and transmission, and the difficulty in properly combining different traits into functional diversity metrics (Cadotte et al. 2011, Hantsch et al. 2014). On the contrary, evolutionary-based metrics such as phylogenetic diversity (the sum of phylogenetic branch lengths of all the species in a certain community) potentially provide alternative ways to quantify ecological differences between species by capturing components of the influence of unmeasured functional traits (Faith 1992, Srivastava et al. 2012, Cadotte et al. 2013). For instance, Gilbert and Webb (2007) found that the probability that a pathogen can infect two plant species decreases with phylogenetic distance between the two plants. In another recent study, Parker et al. (2015) focused on the disease pressure of a single host plant (focal species), and found that disease pressure was best explained by the abundance of all species in the surrounding community, weighted by their phylogenetic distance. However, in those two studies, the effects of phylogenetic diversity on disease were confounded with those of host species abundance and richness. Whether phylogenetic diversity indeed predicts disease emergence and transmission therefore remains unknown. Furthermore, how host phylogenetic diversity affects disease severity at the community level requires much further investigation.

The evidence for and strength of the biodiversity-disease relationship might also be affected by changing environmental constraints. For instance, climate warming can intensify the transmission and severity of fungal pathogens by extending their growth and reproduction times (Ayres 1993, Harvell et al. 2002), whereas the fitness of some fungal pathogens can also be reduced under warming because pathogens vary in their responses to environment conditions (Roy et al. 2004). Analogously, nitrogen fertilization can also have a mixed effect on fungal infections; for example, it can increase disease severity by increasing the concentration of foliar nitrogen, a limiting resource for most foliar fungal pathogens (the nitrogen disease hypothesis; Huber and Watson 1974, Mitchell et al. 2003), or decrease disease severity by enhancing host plant resistance (Huber and Watson 1974, Veresoglou et al. 2013). Indirectly, warming and fertilization can also change the composition and structure of plant communities, reducing species richness while increasing the abundance of some dominant species (Suding et al. 2005), thus affecting pathogen transmission ultimately. However, experimental evidence is currently lacking regarding the complex potential interplays of warming and fertilization on the relationship between biological diversity and disease expression in plant communities. Additionally, there might be interactive effects of fertilization, warming, and host species richness on pathogen load.

We address this lack of evidence for a plant–fungal system in alpine meadows of the Tibetan Plateau. We focus on foliar fungal pathogens (hereafter referred to simply as "pathogens") given that more is known about this group of plant diseases than most others (Fisher et al. 2012), and fungal pathogens are the most important diseases in wild plants of alpine meadows (Zhang 2009). Further, fungal pathogens are largely specific to only one host or to a few closely related species (Gilbert and Webb 2007, Zhang 2009, Gilbert et al. 2012), so they provide an ideal system to test for the drivers of non-vector-borne (aerial and splash-dispersed) diseases. Specifically, we test the following hypotheses: (1) increasing plant (host) diversity as measured by various indices (species richness, evenness, and phylogenetic diversity) changes the severity of foliar fungal diseases, either negatively (dilution) or positively (amplification); (2) different biodiversity indices vary in their capacity to predict fungal infection; (3) artificial warming either increases or decreases the community's susceptibility to fungal disease infection; (4) artificial fertilization (nitrogen addition) increases or decreases the community's susceptibility to fungal disease infection; (5) artificial warming and fertilization together interact to increase or decrease fungal disease infection beyond their simple, additive effects, and (6) the effect of warming and/or fertilization attenuates the relationships between host diversity and fungal disease infection. To test these hypotheses, we developed two experiments: (1) manipulating species richness and phylogenetic diversity by removing specific groups of species in alpine meadow plots; and (2) testing the influences of artificial warming and fertilization on the observed relationships.

Methods

Study site

Our study site was situated in the eastern part of Qinghai-Tibetan Plateau, in Maqu, Gansu Province in the People's Republic of China (101° 53′ E, 35° 58′ N). The experimental alpine meadow is on a southeast-facing gradient at about 3500 m elevation. Mean annual precipitation is 620 mm, most of which falls in the growing season (summer). Mean annual temperature is 1.2°C, with a mean low temperature of −10.7°C in January and high of 11.7°C in July (Liu et al. 2015). The nitrogen-limited soil is typical of alpine meadows. The plant community is dominated by perennial herbaceous species of Poaceae, Ranunculaceae, and Asteraceae, such as *Festuca ovina*, *Kobresia myosuroides*, *Saussurea nigrescens*, *Ligularia virgaurea*, and *Anemone trullifolia.* See Liu et al. (2015) for a complete description of plant species abundance and phylogenetic structure of the community (Appendix S1: Table S1, Fig. S1).

Experimental design

To test our hypotheses, we designed two experiments established in two adjacent meadows in a 100 × 200 m rectangle fenced in 2009, with (yak) grazing only permitted in winter. We did experiment 1 in June 2014, which

consisted of 120 regularly arranged, 1.5 × 1.5 m square plots separated by 3 m from adjacent edges. We selected 12 common herbaceous species with similar abundance to naturally assembled meadows in this site to build our experiments (Liu et al. 2015), which included four grasses, two legumes, and six non-legume herbaceous forbs (Appendix S1: Table S1). Twelve out of 120 plots were maintained as natural assemblages, while the remaining 108 plots were randomly assigned to a species richness treatment of 1 (36 plots, with 3 replicates for each species), 2 (24 replicates), 4 (24 replicates), or 8 (24 replicates). For richness treatment levels of 2, 4, and 8 species, we randomly selected species from the species pool (12 species) while controlling the community phylogenetic diversity (see *Measures of host community diversity*) to cover as a wide a phylogenetic range as possible for different replicates, and then removed all other species by clipping all aboveground parts (and damaging the root as much as possible) appearing in the plot in early June of 2014. We thus created a phylogenetic diversity gradient at each level of species richness. We maintained the monocultures by removing all species except the focal one from the plots in the aforementioned manner. We did a second removal in early July in 2014.

We established the second experiment in June 2011, in which we regularly arranged 60 5 × 5 m plots separated by 1 m from adjacent edges, 12 of which were unmanipulated controls (Appendix S1: Fig. S2). Of the remaining 48, one-half were warmed using transparent, reinforced-plastic, open-top chambers of 1.5 m^2 basal area at the center of the plots and the other one-half were not. These 48 were randomly assigned one of four concentrations of nitrogen addition (NH_4NO_3): 0, 5, 10, or 15 g/m^2, with 6 replicates of each treatment, these concentrations are typical for anthropogenic nutrient deposition in alpine meadows (Li et al. 2014). Thus, there was a total of 18 unmanipulated controls. On average, the open-top chambers increased the air temperature by 0.77°C at night, and by 1.8°C during the day. As for soil (10 cm in depth) temperature, it increased by ~ 0.73°C during the day, and remained nearly unchanged at night. Experiment 2 was manipulated annually from 2011 to 2014 to create a gradient of species richness to occur under fertilization and warming, with species richness not adjusted.

Sampling

For experiment 1, we recorded disease severity (estimated visually using cards with digitized images of leaves of known disease severity, a standard technique in plant pathology) and leaf replicates for each rank (see bottom of this paragraph; Mitchell et al. 2002, 2003) in August 2014 (the peak of the growing season). Recording the disease after two months of removal can ensure the similar abundance of the host species across different species-richness levels. For each host plant species in each plot, we recorded disease severity and visually assessed pathogen group presence (i.e., fungus-caused leaf-spot and blight diseases, rusts, smuts, powdery mildews, and downy mildews) of 25 leaves, with five from each of five randomly selected individuals. For species with no more than five individuals or 25 leaves, we examined all the leaves available. In each plot, we also randomly arranged three 0.2 × 0.5 m subplots parallel to one edge of the plot at least 0.1 m away from either edge, harvested all the stems in each quadrat at ground level, sorted to species, recorded each species, abundance, and then dried and weighed them to 0.1 mg.

For experiment 2, we measured disease severity the same way as in experiment 1, additionally recording species composition, abundance, and dry biomass in a 0.5 × 0.5 m subplot centred in each plot.

We distinguished fungal leaf-spot and blight diseases from other spot or blight diseases using the following protocol. According to plant pathology methods, there are specific symptoms of fungal pathogen that can be distinguished from other plant consumers (i.e., bacteria, viruses, insects). In our study site, fungal diseases inevitably have the following two characteristics: (1) there must be lesions present in the plant of different shapes and (2) there are differently colored mold shapes or powder on the lesions (diseased leaves). In contrast, other plant consumers have different characteristics: (1) insect damage consists of mechanical damage to the plant leaves and (2) neither bacterial nor viral diseases have associated molds or powder on the lesions of diseased leaves, with viral diseases additionally causing leaf deformation (Zhang 2009). We also collected three samples of infected plant tissue per plant species and pathogen group in the same study site in July 2015 and confirmed the taxa of the pathogens (fungal, bacteria, or viral diseases) in the lab using an OLYMPUS light microscope (Olympus Corporation, Tokyo, Japan).

Measures of disease severity

We defined a severity index (V_i) as the average proportion of leaf area of the specific plant species infected by disease i, and pathogen diversity (S_p) as the cumulative number of pathogen groups per plant species and plot (Rottstock et al. 2014).

We also calculated the community-weighted means of V, which is calculated the same as the community pathogen load (l) (Mitchell et al. 2002, 2003):

$$l = \frac{\sum_{i=1}^{S} a_i \times V_i}{\sum_{i=1}^{S} a_i}$$

where S is the total number of diseases and a_i is the abundance of plant species specific to the ith disease. Pathogen load (l) has been widely used in plant disease ecology and is considered to be a good proxy of disease infection (e.g., Mitchell et al. 2002, 2003, Hantsch et al. 2013, 2014).

Measures of host community diversity

From the abundance and richness data, we calculated host species richness (S_h) and Shannon's evenness index

(H'_h) for host plant evenness using the function *diversity* in package *vegan* (Oksanen et al. 2013). A full description of the method used to estimate community phylogenies is provided in Liu et al. (2015), but we provide a brief synopsis here (see Appendix S1: Fig. S1 for the phylogenetic tree of all the species in the study site). To avoid similar branch lengths among some species, we used the *compute.brlen* function in package *ape* (Paradis et al. 2004) to compute branch lengths of the tree using Grafen's methods (Grafen 1989), and then calculated several measures of phylogenetic diversity using the *picante* package (Kembel et al. 2010): Faith's PD (PD hereafter), which is the sum of phylogenetic branch lengths (Faith 1992), and mean pairwise distance (MPD), which is the average distance separating all pairs of species on the phylogenetic tree (Webb et al. 2002).

Analysis

To assess the relationship between pathogen load and host diversity (including species richness, evenness, and phylogenetic diversity), we constructed a series of generalized linear models. We calculated the Spearman rank-order correlation between indices we considered using the *cor.test* function in the *stats* package to identify highly correlated variables (Appendix S1: Tables S2 and S3). We validated the use of a gamma distribution of modelled error distribution based on the normalized scores of standardized residual deviance.

Our model-building protocol was to construct a full-subsets model set for experiment 1 (i.e., all combinations of the three diversity metrics S_h, H'_h, and PD). For experiment 2, however, we split the model sets into two phases: (1) first considering the three diversity metrics as single-term models to determine whether the explanatory power and rank of diversity metrics changed as a result of the warming and fertilization manipulations, and (2) then including the most parsimonious (i.e., greatest explanatory power for the fewest number of predictors according to information-theoretic model-weighting criteria [see next paragraph]) diversity metric from phase 1 into models considering the effects of warming (W) and nitrogen fertilization (N; including their interaction $N \times W$).

We calculated the information-theoretic Akaike's information criterion corrected for small sample sizes (AIC_c) to evaluate relative model support. We also calculated percent deviance explained in the response variable (De) as an index of each model's goodness-of-fit. To test the dilution effect for each disease in natural plant assemblages, we selected the disease severity index (V_i) of the target disease as the response variable and the number of cooccurring plot species as the independent variable in linear models for experiment 1. We used the information-theoretic evidence ratio (ER, $w\text{AIC}_c$ [slope model]:$w\text{AIC}_c$ [intercept-only model]) as an index of relative support for the linear slope model. We did all analyses using R 2.15.1 (R Development Core Team 2014).

Results

We found no evidence for a relationship between species richness in a plot and the abundance of each host species across richness levels in experiment 1 (information-theoretic evidence ratio, $ER < 1$; Appendix S1: Table S4); therefore, abundance effects can be ignored when considering the relationship between host plant diversity and foliar fungal disease. In both experiments, fungal pathogen diversity increased with host species richness (Fig. 1). In experiment 1, 30 of the disease severity (V_i) relationships could be tested (i.e., sufficient degrees of freedom); of these, 20 (67%) had higher ($ER > 1.5$) support for a negative slope model (i.e., dilution effect), whereas only 1 (3%) had support for a positive relationship (i.e., amplification effect) between severity and

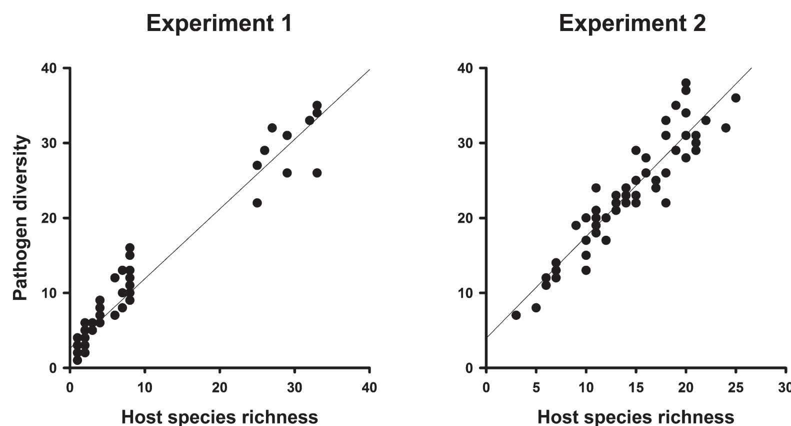

Fig. 1. Linear increase of pathogen diversity (the cumulative number of pathogen groups per plot) with increasing host species richness in both experiment 1 (species richness and phylogenetic diversity were manipulated through removal; information-theoretic evidence ratio [ER] = 4.08×10^{75}; percentage of deviance explained [De] = 94.74) and experiment 2 (experimental warming and nitrogen fertilization; $ER = 5.47 \times 10^{22}$; De = 86.20).

Table 1. Generalized linear model (GLM, family = gamma, link = log) results for pathogen load (l) as a function of host species richness (S_h), Shannon's evenness index for host (H'_h), phylogenetic diversity of host plant community (PD), warming treatment (W), nitrogen fertilization treatment (N), and combination of nitrogen fertilization and warming treatment ($N \times W$).

Model	LL	k	AIC_c	ΔAIC_c	$wAIC_c$	De
Experiment 1						
$\sim S_h$ + PD	−381.961	3	772.267	0	0.322	43.7
$\sim H'_h$ + PD	−382.176	3	772.696	0.429	0.260	43.5
~PD	−383.484	2	773.173	0.906	0.205	42.3
$\sim S_h + H'_h$ + PD	−381.349	4	773.219	0.952	0.200	44.2
$\sim S_h + H'_h$	−385.663	3	779.671	7.404	0.008	40.3
$\sim H'_h$	−387.153	2	780.511	8.244	0.005	38.9
$\sim S_h$	−396.927	2	800.060	27.793	<0.001	28.8
~1 (null)	−419.088	1	842.278	70.011	<0.001	—
Experiment 2 (Phase 1)						
$\sim S_h$	−147.961	2	302.401	0	0.937	37.4
$\sim H'_h$	−150.706	2	307.891	5.490	0.060	30.9
~PD	−153.693	2	313.865	11.464	0.003	23.0
~1 (null)	−160.976	1	326.188	23.786	<0.001	—
Experiment 2 (Phase 2)						
$\sim S_h + N$	−145.239	3	299.295	0	0.354	15.7
$\sim S_h + N + N \times S_h$	−144.078	4	299.405	0.111	0.335	22.0
$\sim S_h + N + W$	−145.140	4	301.530	2.235	0.116	16.3
$\sim S_h$ (null)	−147.961	2	302.401	3.107	0.075	—
$\sim S_h + N + W + N \times W$	−144.312	5	302.412	3.117	0.074	20.7
$\sim S_h + W$	−147.519	3	303.853	4.559	0.036	2.7
$\sim S_h + W + W \times S_h$	−147.515	4	306.279	6.984	0.011	2.7

Notes: For experiment 1, we included all possible combinations and the intercept-only (null) model. For experiment 2, phase 1 examines the relative support for single-term models only, and phase 2 incorporates the best predictor from phase 1 with N, W, and $N \times W$ (here, the null model is the one including only the diversity term). Shown are the estimated number of model parameters (k), maximum log-likelihood (LL), the information-theoretic Akaike's information criterion corrected for small samples (AIC_c), change in AIC_c relative to the top-ranked model (ΔAIC_c), AIC_c weight ($wAIC_c$ = model probability) and the percentage of deviance explained (De) as a measure of the model's goodness-of-fit; "—" in De means no value is available for the null model.

host species richness. The remainder (9; 30%) had weak or no evidence ($ER < 1.5$) for the slope model (i.e., no evidence for an effect; Appendix S1: Table S5).

In experiment 1, community pathogen load (l) was negatively related to plot-level plant species richness, plant species evenness, and plant phylogenetic diversity (Table 1; Fig. 2). Furthermore, in all of the generalized linear models constructed to explain variation in pathogen load in experiment 1, the model consisting of phylogenetic diversity alone outperformed all the other diversity metrics (including species richness) based on the model rankings ($wAIC_c$ = 0.205), which by itself accounted for > 42% of the deviance explained in pathogen load, with either host species richness or evenness having little additional explanatory power in terms of deviance explained (Table 1). After controlling possible confounding effects of species richness with phylogenetic diversity, we found consistent, negative relationships between phylogenetic diversity and pathogen load at the species richness levels of 2, 4, and 8 (Fig. 3; Appendix S1: Table S6), respectively.

In experiment 2, the range of species richness in the subplots varied from 3 to 25 after four years of experimental warming and nitrogen fertilization (Fig. 1, x-axis). Both experimental warming and nitrogen fertilization enhanced pathogen load (Appendix S1: Fig. S3). With experimental warming and fertilization (experiment 2), community pathogen load was negatively related to plot-level plant species richness, plant species evenness, and plant phylogenetic diversity (Table 1; Fig. 2). However, the most parsimonious predictor of pathogen load shifted to host species richness (Table 1; phase 1). This difference might have arisen because there was a positive relationship between species richness and mean pairwise difference in the removal treatments (experiment 1, ER = 2.88), while mean pairwise difference was negatively related to species richness under warming and/or fertilization (experiment 2, ER = 15.38; Appendix S1: Table S7, Fig. S4).

We therefore included host species richness in the generalized linear models testing for the effects of warming and fertilization. After taking host richness into account (i.e., setting host richness as the null model), nitrogen fertilization had the highest additional explanatory power (De = 15.7%), followed by a weaker effect of warming (De = 2.7%). There was also some support for the interaction between nitrogen fertilization and warming increased pathogen load (Table 1); however, there was a stronger interaction between host richness and nitrogen fertilization (fertilization weakened the

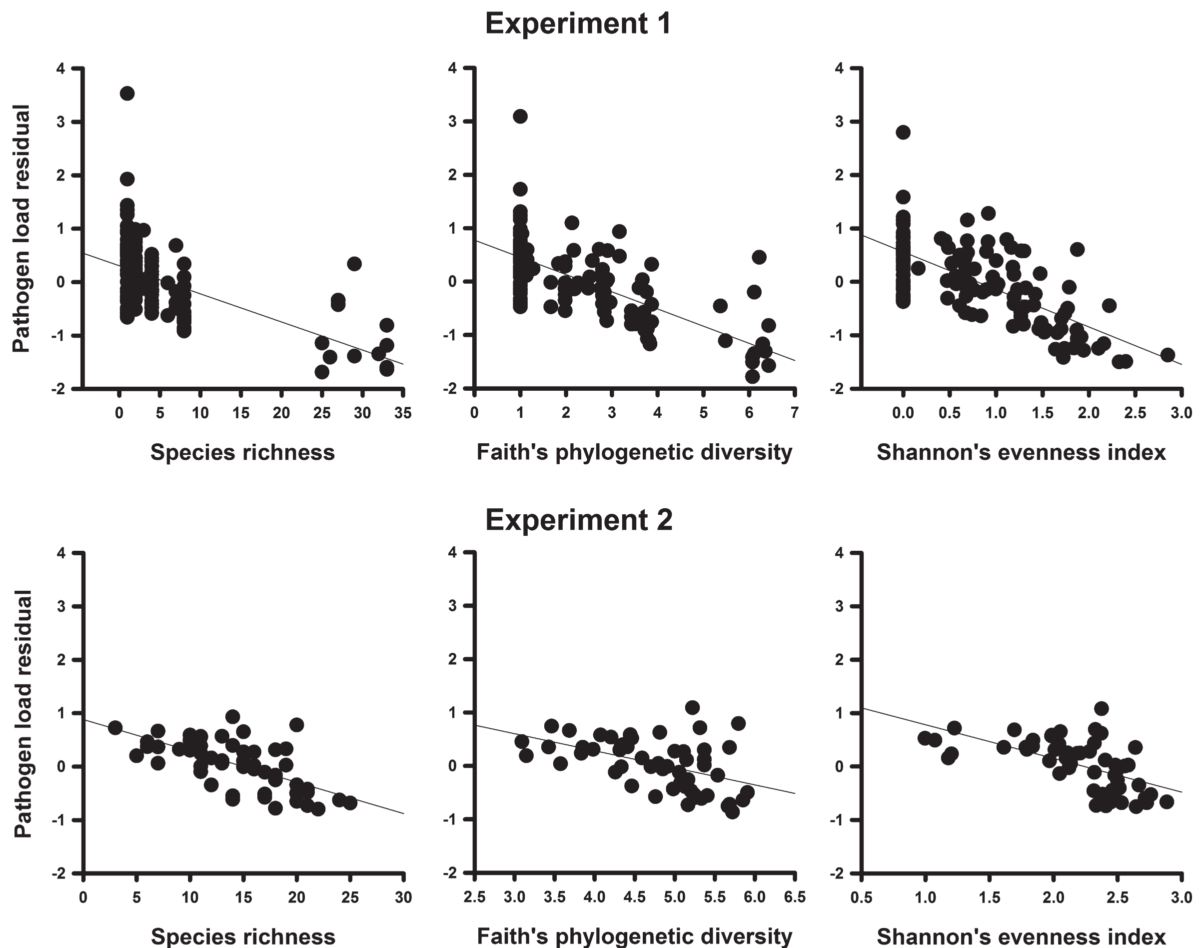

Fig. 2. Partial residual plots of each single diversity index in the generalized linear models to predict variation in pathogen load. Species richness and phylogenetic diversity were manipulated through removal in experiment 1, whereas experiment 2 refers to experimental warming and nitrogen fertilization.

negative relationship between pathogen load and host species richness; Fig. 4).

Discussion

Using a removal experiment that manipulated species richness and host community phylogenetic diversity in natural assemblages, we detected a dilution effect rather than an amplification effect of biodiversity on foliar fungal disease severity, while controlling for equal abundance of different plant species and irrespective of richness level. This dilution effect arose despite increased pathogen diversity with host diversity, which is consistent with the general biodiversity–disease relationship expected to operate in natural communities (Mitchell et al. 2002, Cardinale et al. 2012, Johnson and Hoverman 2012, Rottstock et al. 2014).

Most previous investigations of this type of dilution effect attribute the phenomenon to compensatory declines in host abundance with increasing host diversity (Knops et al. 1999, Zhu et al. 2000, Mitchell et al. 2002, 2003, Roscher et al. 2007, Rottstock et al. 2014). As such, one would predict the negative relationship between diversity and disease to disappear for most diseases once abundance effects are removed (Mitchell et al. 2002, 2003), because disease transmission is generally related to host abundance (Burdon and Chilvers 1977, Chapin et al. 1997, Mitchell et al. 2002, 2003). However, even when we equalized host abundance across different species richness levels in the removal experiment (experiment 1), the negative relationship persisted. Hence, we can conclude that the dilution effect arises mainly in association with enhanced diversity itself rather than from shifting abundances. Our results therefore match theoretical expectations that a dilution effect can occur if the host community is additive (i.e., increasing host richness with no compensatory declines in host abundance; Rudolf and Antonovics 2005).

The mechanisms leading to a dilution effect could be from physical isolation and resistance enhancement. On the one hand, because of the intensive evolutionary arms race between host plants and fungi, most fungal

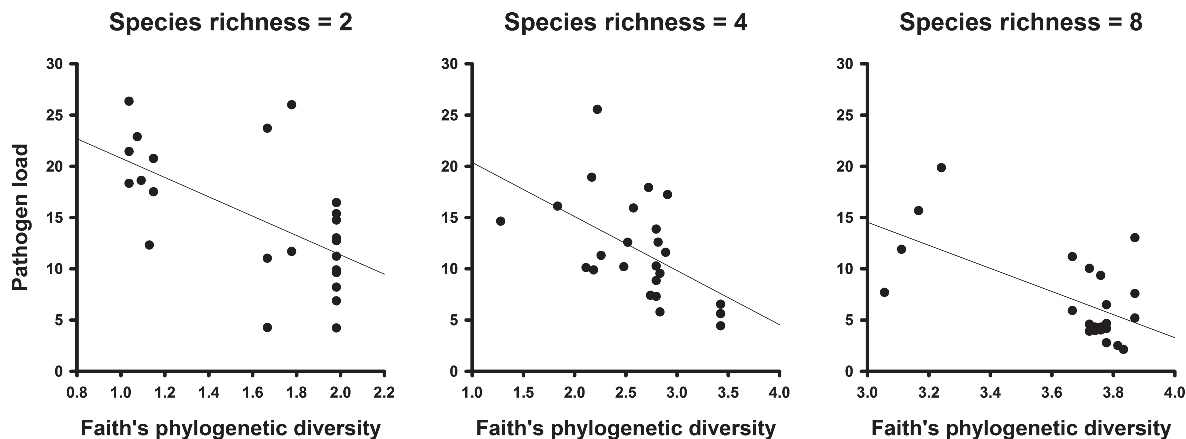

Fig. 3. Linear decrease of pathogen load (*l*) with increasing phylogenetic diversity of host plant community (PD) at species richness levels of 2 (information-theoretic evidence ratio [*ER*] = 49.59; percentage of deviance explained [*De*] = 35.26), 4 (*ER* = 13.43; *De* = 27.82), and 8 (*ER* = 37.48; *De* = 37.48) in experiment 1, where species richness and phylogenetic diversity were manipulated through removal.

pathogens are generally specific to only one host or a few closely related host plants in our study site (Zhang 2009); hence, increasing host plant species richness is predicted to increase the interception of spores by non-hosts (physical isolation; Trenbath 1977, Mitchell et al. 2003). On the other hand, because the abundances of the host species were equal across different species-richness levels, the total abundance/cover, spatial heterogeneity and three-dimensional space-filling capacity would increase with increasing species richness (Rottstock et al. 2014). Therefore, increasing host species richness should also alter micro-climatic conditions (e.g., temperature, humidity, illumination, raindrop splash), resulting in reduced average pathogen load (Zhu et al. 2000, Ostfeld and Keesing 2012). Furthermore, higher plant diversity consistently led to higher pathogen diversity under natural, warming, and high-nitrogen conditions, because the diversity of higher trophic levels strongly depends on the diversity of their hosts, through both host availability and evolutionary covariation (Chapin et al. 1997, Kamiya et al. 2014). This is also consistent with previous results for parasitic fungal pathogen diversity and infection in experimentally assembled grasslands (Rottstock et al. 2014). The existence of multiple pathogens might also induce resistance because of incompatible interactions between obligate parasitic fungal pathogens, a phenomenon known as “cross-protection” in phytopathology. Such a mechanism potentially contributes to the relatively lower pathogen load observed in diverse assemblages (e.g., Zhu et al. 2000, Rottstock et al. 2014).

Unlike recent studies experimentally confounding the effects of phylogenetic diversity and species richness on disease expression (Gilbert and Webb 2007, Parker et al. 2015), for the first time we show that phylogenetic diversity itself dampens foliar fungal disease in plants at different levels of species richness (i.e., phylogenetic dilution effect). It has been recently suggested that the experimental effect of variation in phylogenetic diversity on ecosystem function is frequently confounded with species richness, and that the relationship to changes in phylogenetic diversity would disappear after controlling for variation in richness (Venail et al. 2015). In this regard, our study provides strong experimental support for the importance of phylogenetic diversity on ecosystem function and services such as disease suppression within a community.

We found that phylogenetic diversity was the most effective predictor of variation in the severity of foliar fungal pathogens in natural assemblages. The higher predictive capacity of phylogenetic diversity compared to other biodiversity metrics in explaining the variation in pathogen load potentially arises because it more completely synthesizes both known and unmeasured functional traits that influence pathogen transmission, host susceptibility and immune responses (Gilbert and Webb 2007, Srivastava et al. 2012). This phenomenon might eventuate because greater phylogenetic diversity implies greater physical isolation, such that the probability a pathogen will infect a host decreases as the phylogenetic distance between two host plants widens (Gilbert and Webb 2007, Gilbert et al. 2012, Parker et al. 2015).

As expected, we found that artificial fertilization weakened the diluting effect of increasing host species richness on pathogen load, most likely by enhancing fungal spore production, infection success, and lesion growth by the hosts (Mitchell et al. 2003, Veresoglou et al. 2013). Artificial warming, on the other hand, slightly increased pathogen load via associated changes in plant phenology and physiology, as well as soil drying and other biogeochemical changes (Roy et al. 2004). Nitrogen fertilization might benefit fungal growth more directly than artificial warming, thus leading to a relatively weaker effect of the latter. The weak positive relationship between warming and pathogen load became weakly negative after removing the relatively strong effect of fertilization, host species richness, and the interaction of

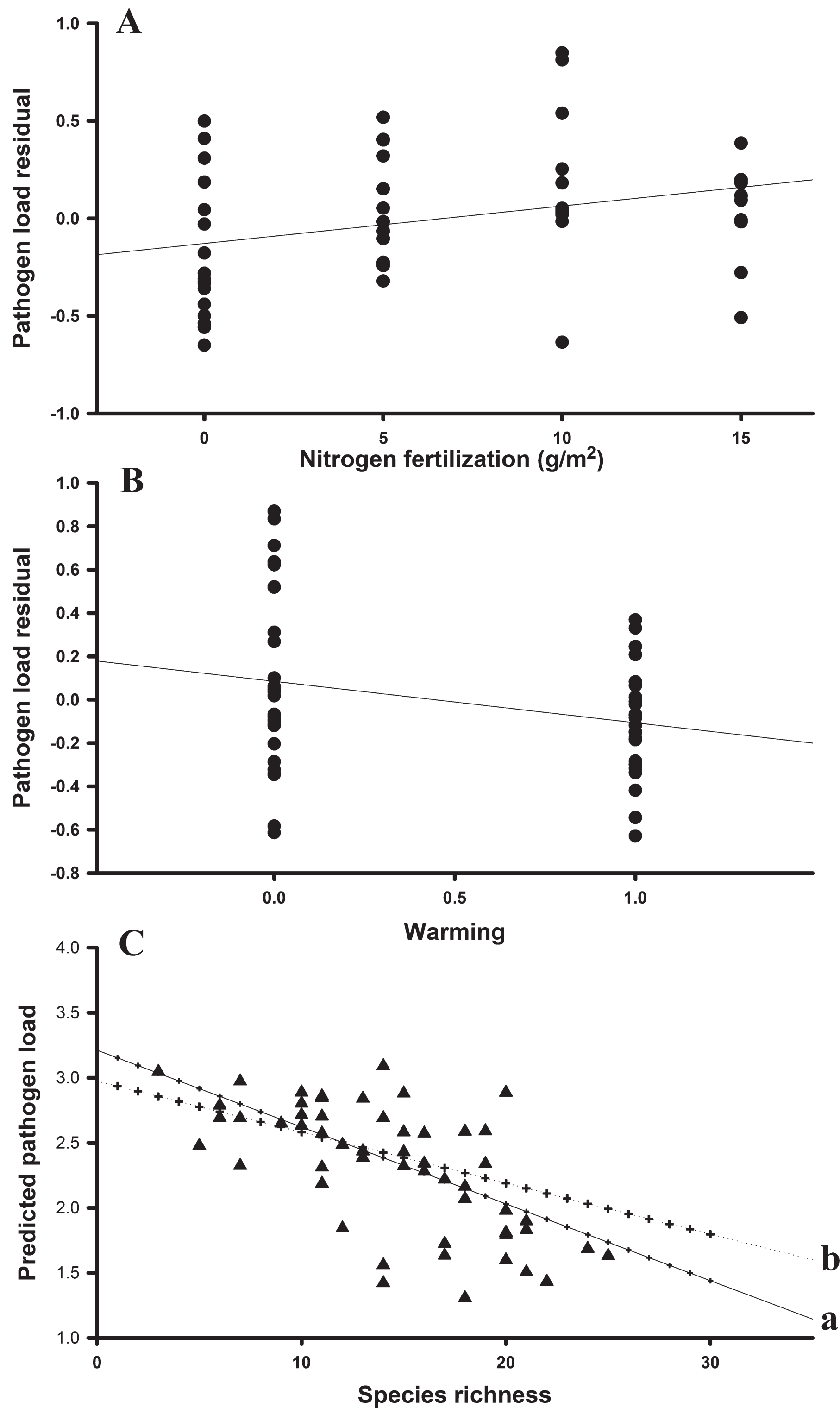

FIG. 4. Partial residual plots in the generalized linear models to predict variation in pathogen load in experiment 2. (A) Partial residuals for nitrogen fertilization from the saturated model ($l \sim S_h + N + W + N \times W$), (B) Partial residuals for warming from the saturated model ($l \sim S_h + N + W + N \times W$). (C) Nitrogen fertilizations weakens the relationship between species richness and pathogen load: (a) prediction of pathogen load (l) with host species richness (S_h) from the $l \sim S_h$ model in experiment 2; (b) predicted pathogen load when keeping nitrogen fertilization constant at 6.67 g/m^2 (average nitrogen addition across treatments in experiment 2) with species richness from the $l \sim S_h + N + N \times S_h$ model in experiment 2. Small triangles indicate the raw values measured in experiment 2. Model factors are described in Table 1.

fertilization and warming. In addition, we found an interaction between host richness and nitrogen fertilization (additional 6.7% deviance explained in pathogen load), meaning that the pathogen load of species-rich communities was more sensitive to nitrogen fertilization than that of species-poor communities, and thus confirming the attenuation of the dilution effect.

Experimental warming and fertilization also changed the relationship between mean pairwise distance and species richness from positive to negative. As such, the degree to which phylogenetic diversity decreased as species richness declined was not consistent across experiments. Instead, loss of species from warming and fertilization resulted in the community becoming more phylogenetically overdispersed; therefore, we would expect lower pathogen infection because of the increased physical isolation among these phylogenetically less-related hosts (Gilbert and Webb 2007, Gilbert et al. 2012). In other words, the phylogenetically overdispersed community, which implies low spore transmission efficiency, offsets the effects of varying diversity, and leads to reduced explanatory power.

Compared to planting experiments where artificial communities are constructed from scratch, our removal experiment more realistically mimics natural community composition and its assembly mechanisms, which is critical for disease transmission (Dìaz et al. 2003, Liu et al. 2015). However, the removal experiment could also disturb community structure and hence, modify disease severity. Both removal and planting experiments, therefore, have advantages and disadvantages for investigating biodiversity–disease relationships (Rottstock et al. 2014). We conclude that the intensity of the dilution effect of host biodiversity on disease severity can vary according to other external pressures experienced by the community. Even for a specific pathogen–host system, there is no consistent biodiversity metric that can best explain variation in disease severity under different scenarios. The relative power of different biodiversity metrics to explain the severity of pathogens might instead depend on the relationship between different metrics, emphasizing the importance of experimentally separating and evaluating the effects of different biodiversity metrics on ecosystem functioning and services.

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