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Species decline under nitrogen fertilization increases community-level competence of fungal diseases

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The artificial fertilization of soils can alter the structure of natural plant communities and exacerbate pathogen emergence and transmission. Although the direct effects of fertilization on disease resistance in plants have received some research attention, its indirect effects of altered community structure on the severity of fungal disease infection remain largely uninvestigated. We designed manipulation experiments in natural assemblages of Tibetan alpine meadow vegetation along a nitrogen-fertilization gradient over 5 years to compare the relative importance of direct and indirect effects of fertilization on foliar fungal infections at the community level. We found that species with lower proneness to pathogens were more likely to be extirpated following fertilization, such that community-level competence of disease, and thus community pathogen load, increased with the intensity of fertilization. The amount of nitrogen added (direct effect) and community disease competence (indirect effect) provided the most parsimonious combination of parameters explaining the variation in disease severity. Our experiment provides a mechanistic explanation for the dilution effect in fertilized, natural assemblages in a highly specific pathogen–host system, and thus insights into the consequences of human ecosystem modifications on the dynamics of infectious diseases.

1. Introduction

Ecological systems and the services they provide are continuing to be degraded by human development and endeavour [1,2]. One of these modifications receiving increased research attention is the impact of eutrophication via the deposition of nitrogen from artificial fertilization on species identity, biodiversity patterns, ecosystem functions, and disease risk in plants [3,4]. Although the effects of nitrogen fertilization on biodiversity [5–9], community productivity [3,10], stability [11], and nitrogen cycles in plant communities [12] are well documented, few studies have examined the community-level changes arising from the effect of fertilization on a community's capacity to support diseases [13]. Fertilization can potentially affect a plant's disease risk either directly or indirectly through changes in community structure or nitrogen concentration [14]. However, the relative strength of these different pathways to modify disease severity (primarily fungal diseases) remains largely uninvestigated.

Despite mixed conclusions regarding the direct effect of nitrogen fertilization on plant disease severity, the dominant contention is that it generally increases the severity of foliar fungal diseases [4,15]. High nitrogen availability can increase a plant's proneness to fungal pathogens because of increased foliar nitrogen concentration, which is a limiting resource for most such pathogens (the 'nitrogen-disease' hypothesis) [14,15]. Based on a meta-analysis of 57 studies, Veresoglou *et al*. [4] found a positive relationship between fertilization and disease severity in non-crop dicots, but a weak, negative relationship

emerged for non-crop grasses. Other evidence suggests that adding nitrogen can also decrease disease severity by enhancing host plant resistance [16]. Thus, the direct, net effect of fertilization on foliar fungal disease depends on the balance between these two processes.

Besides altering individual plant susceptibility, adding nitrogen can also change the structure of plant communities [6,17], affecting pathogen transmission and prevalence at the community level [18]. Thus, individual plants might also experience changes in disease severity merely by virtue of the emergent biodiversity patterns in their immediate community. There are two main mechanisms to explain changes in disease prevalence at the community level: (i) the exclusion of competitively inferior species and/or (ii) the sequential loss of 'competent' hosts (*host competence* describes the potential for hosts to affect the dynamics of generalist pathogens, which depends *inter alia* on host susceptibility and the form of pathogen transmission). Many nitrogen-fertilization field experiments and observational studies have demonstrated that fertilization can lead to a decline in biodiversity by hindering competitively inferior species, especially in grasslands [5,7,19]. Because disease transmission generally depends on host abundance [18,20], increasing host diversity often leads to a reduction in the abundance of many constituent species, in turn, reducing the overall prevalence of disease (the 'dilution' effect) [13,14,18,21–24]. Previously, Liu *et al*. [13] found that artificial fertilization weakened the diluting effect of increasing host species richness on disease severity; however, the underlying mechanisms remain unknown.

It has been demonstrated that adding limiting resources reduces niche dimensionality and eliminates potential trade-offs, thus reducing the number of plant species that can coexist [25]. In this case, species exclusion might be tied to asymmetric competition favouring some species with specific traits (e.g. plant height and leaf nitrogen content), leading to a functional-based loss of species [3]. There is also evidence for a negative relationship between host 'competence' for generalist pathogens (the ability to host and propogate generalist pathogens) and the order of species loss under land-use intensification [1,26]. Here, highly competent hosts dominate in low-diversity host communities, thus more efficiently perpetuating pathogens, whereas non- or poorly competent host species are more likely to persist in high-diversity communities. Thus, highly diverse communities should have a lower net community competence (i.e. the 'ubiquity–competence' relationship), which might be an underlying mechanism that promotes the dilution effect. A positive relationship between host ubiquity and competence might be attributable to evolutionary trade-offs among host competitive ability, growth, resilience, and disease resistance [27,28], because both competition and defence are energetically costly (especially for defending against specialist enemies) [28–30]. Hence, the least competent host species might be the most at risk of loss under fertilization as a consequence of their lower competitive ability (according to a functional-based mechanism). However, few experiments have explicitly tested whether this relationship exists in specific pathogen–host systems, and whether the predictions might change depending on the nature of the pathogen–host systems under investigation.

To address this knowledge gap, we implemented a 5-year nitrogen-addition experiment in an alpine meadow of the Tibetan plateau to test the relative roles of both direct and indirect mechanisms on the severity of foliar fungal diseases at the community level. We focused on foliar fungal pathogens (hereafter 'pathogens') given that these are the most important diseases in wild plants of Tibetan alpine meadows [31]. Further, fungal pathogens are largely specific to a particular group (family or genus) or single hosts, so they provide an ideal system to test for the effects of nitrogen fertilization on relatively specialist pathogens. We measured host plant community structure along a gradient of fertilization over 5 years, and assessed the relative magnitude of the direct and indirect mechanisms to test the following hypotheses: (i) an evolutionary trade-off mechanism underlies the fertilization-driven sequence of species loss. Here, we expect that fertilization favours species with higher maximum height (that are better light competitors), low nitrogen content (reduced competition for soil resources—e.g. nitrogen-fixing species such as leguminous forbs lose their competitive advantage once nitrogen is no longer a limiting resource), and higher competence (more prone to disease), (ii) community competence increases with the intensity of nitrogen addition, (iii) decreasing host species richness following fertilization increases the severity of foliar fungal diseases (dilution effect), (iv) the combination of the amount of nitrogen added (direct effect) and the community competence index (indirect effect—a high community competence index indicates that it has a high capacity to support infection; see below for the method of its calculation), rather than either of these effects alone, explains the most variation in the severity of foliar fungal pathogens.

2. Material and methods

(a) Study site

We carried out our field experiment in the eastern part of the Qinghai–Tibetan plateau, in Maqu, Gansu Province, China (101°53′ E, 35°58′ N; 3 500 m above sea level). The mean annual temperature is 1.2°C, with the monthly average ranging from −10.7°C in January to 11.7°C in July. There are more than 270 days year^{-1} of frost. The mean annual precipitation is 620 mm, 85% of which falls during the short growing season (June–September). The nitrogen-limited soils are classified as 'chestnut' or 'alpine meadow' soils with a mean thickness of 80 cm [32]. The grassland vegetation is a typical alpine meadow, and is dominated by perennial herbaceous species of Poaceae, Asteraceae, and Ranunculaceae, such as *Anemone trullifolia*, *Festuca ovina*, *Kobresia myosuroides*, *Ligularia virgaurea*, and *Saussurea nigrescens* (see electronic supplementary material, table S1 for the entire species list). The dominant animals include yaks, horses, marmots (*Marmota himalayana*), zokor (*Myospalax* spp.), and ants [33].

(b) Experimental design

In June 2011, we established our experiment on a southeast-facing meadow with a little slope that was fenced in 2009, with grazing (mainly yaks) only permitted during winter. We regularly arranged 60 5 × 5 m plots (including 12 additional, non-manipulated plots) separated by 1 m from adjacent edges with roughly the same species diversity and community structure. We randomly assigned the remaining 48 plots one of four concentrations of nitrogen addition (supplied as ammonium nitrate NH_4NO_3, which was a short-term release of nitrogen into soil): 0 (control), 5, 10, or 15 g m^{-2}, with 12 replicates of each treatment. We broadcast the fertilizer evenly over the plot

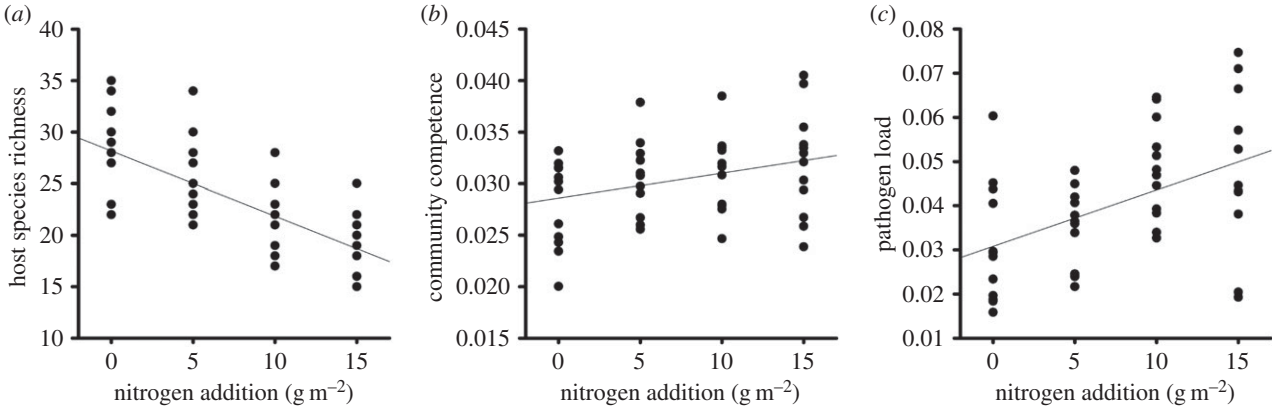

Figure 1. Linear relationship between host species richness, pathogen load, community competence, and nitrogen addition of 48 main experimental plots in 2015 (there are some overlapping points in this figure). (*a*) Host species richness (information-theoretic evidence ratio [ER] = 1.31×10^6; percentage deviance explained [*De*] = 46.99). (*b*) Community competence (ER = 4.33; *De* = 10.29); a strong positive effect of nitrogen addition on community competence means a change in community structure under fertilization towards highly disease-competent species. (*c*) Pathogen load (ER = 178.03; *De* = 23.16).

once per year in mid-June (the early stage of the growing season) from 2011 to 2015 during overcast conditions (because exposure to direct sunlight degrades surface nitrogen more quickly) to create a large change in community structure of the fertilized assemblages (figure 1*a*). Fertilization once a year was sufficient to alter community structure because of the short, four-month growing season.

(c) Sampling

In August (the peak of the growing season) 2015, by avoiding the 1.5 m^2 area at the centre of plots (6% of the plot's total area; where transparent, reinforced-plastic, open-top chambers were arranged for artificial warming in the growing season from 2011 to 2014 for another study [13] in half of the plots), we randomly arranged a 0.5×0.5 m subplot parallel to one edge of each plot at least 1 m away from any plot's edge. We harvested all the stems in each subplot at ground level, sorted to species, recorded each species' abundance (measured as the number of individuals found per sample), then dried the samples at 70°C and weighed them to 0.1 mg.

We recorded disease severity (estimated visually using cards with digitized images of leaves of known disease severity, a standard technique in plant pathology) on leaf replicates (see below) in August 2015 [13,18]. For each host plant species in each plot, we recorded disease severity and visually assessed the presence of pathogen groups (i.e. fungus-caused leaf-spot disease and blights, rusts, smuts, powdery mildews, and downy mildews) of 25 leaves, with five from each of five randomly selected individuals. For species with less than or equal to five individuals or less than or equal to 25 leaves, we examined all the leaves available. To obtain enough samples to calculate the disease proneness index (see below), we also recorded disease severity with the same approach for an additional 20 natural plots (0.5×0.5 m; spaced 10 m from each other) adjacent to the fertilization experiment. Hence, a total of 32 natural plots in our study site were used to calculated a disease proneness index. According to standard plant pathology methods, there are specific symptoms of fungal pathogens that can be distinguished from other plant consumers. A full description of the protocols used to distinguish fungal diseases is provided in Liu *et al.* [13], but we provide a brief synopsis here: (i) there must be lesions present on the plant of different shapes and (ii) there are differently coloured mould shapes or powder on the diseased leaves [13,31], whereas other plant consumers or physiological deficiencies have different characteristics. We also collected three samples of infected plant tissue per plant species in the same study site in July 2015 and confirmed the groups of pathogens (i.e. fungus-caused leaf-spot disease and blights, rusts, smuts, powdery mildews, and downy mildews) in the laboratory using an OLYMPUS light microscope (electronic supplementary material, table S1).

(d) Measures of disease severity

We defined a disease 'severity' index (V_i) as the average proportion of leaf area of the specific plant species infected by disease i (e.g. 0.01 means 1% of leaf area infected by diseases) and calculated the community-weighted means of V, which is equivalent to 'community pathogen load' (l) [14,18]:

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

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 a good indicator of infection severity [34,35].

Within fertilization treatments, communities differed in structure (the identity of certain species present), which could influence pathogen load. To test how much variation in pathogen load was explained by the variation in species composition between plots and to avoid any confounding effects of fertilization, we defined a 'disease proneness index' (hereafter referred to as 'P_i') as the average severity index (V_i) in the 32 natural plots (12 controls plus 20 additional natural plots) of the specific plant species infected by disease i. We then calculated a 'community competence index' (hereafter referred to as 'p') for each plot by calculating a host abundance-weighted average of the P_i over all fungal diseases per plot [1,18]:

$$p = \frac{\sum_{i=1}^{S} a_i P_i}{\sum_{i=1}^{S} a_i},$$

where p is considered a reasonable indicator of community-level P_i as well as the capacity to support fungal diseases—a high p means a high community capacity to support infection [1].

(e) Host trait data and community diversity

We measured seven plant traits generally considered important for plant-to-plant interactions for 54 of the total 63 species (these 54 species constitute more than 99% of the individuals per plot) only in natural conditions in the study site: (i) average and (ii) maximum plant height (cm), (iii) leaf nitrogen, and (iv) phosphorus content (mg g^{-1}), (v) *per capita* seed mass (mg), (vi) specific leaf area (cm^2 g^{-1}), and (vii) N–P ratio [36]. A full

Table 1. Results of linear models testing the relationship between nitrogen fertilization (g m^{-2}) and seven community-weighted mean (CWM) functional traits of 48 main experiment plots in 2015. Shown are the number of model parameters (*k*), slope (β_1), intercept (β_0), information-theoretic Akaike's information criterion corrected for small samples (AIC_c), change in AIC_c relative to the intercept-only model (ΔAIC_c), AIC_c weight ($wAIC_c$ = relative model probability), the evidence ratio (ER, $wAIC_c$[slope model] : $wAIC_c$[intercept-only model]), and per cent deviance explained (*De*). The seven functional traits were derived from natural plots; this table shows a change in species composition under fertilization towards species that are taller, richer in leaf phosphorus content, poorer in leaf nitrogen content, better producers of seed, and with lower N–P ratios.

CWM traits	*k*	β_1	β_0	AIC_c	ΔAIC_c	$wAIC_c$	ER	*De*
average height	46	0.264	21.299	237.816	10.355	0.994	177.230	23.1
max height	46	0.279	34.717	263.518	6.349	0.960	23.912	16.5
leaf P	46	0.003	0.209	−164.473	5.990	0.952	19.987	15.8
leaf N	46	−0.016	2.439	−70.167	22.362	1.000	71 765.900	40.2
seed mass	46	0.013	1.138	−2.412	3.027	0.820	4.542	10.5
specific leaf area	46	0.397	182.937	411.804	−1.415	0.330	0.493	1.8
N–P ratio	46	−0.189	11.726	188.978	14.805	0.999	1639.669	30.0

description of the methods used to collect these plant traits is provided in Liu *et al.* [33].

For each subplot, we calculated host species richness (S_h) and Shannon's evenness index (H'_h) for host plant evenness, using the function *diversity* in the R package `vegan` [37]. Further, we quantified the effects of fertilization on community structure by calculating community-weighted means (CWM) of plant functional trait values (*t*) weighted by host species abundances within a certain community:

$$\mathrm{CWM} = \frac{\sum_{i=1}^{S} a_i t_i}{\sum_{i=1}^{S} a_i}.$$

Therefore, in this case, a higher community-weighted mean functional traits index means a change in community structure of treatment plots towards species that are taller, richer in leaf phosphorus and nitrogen content, better producers of seed, and with higher N–P ratios compared with 12 control plots (0 g m^{-2}), respectively.

(f) Analysis

We determined the slope of the nitrogen–abundance relationship for each species (based on both raw plant species abundances and relative abundances), with the abundance of a focal species as the response variable and the amount of nitrogen addition as the independent variable in linear models. The slope parameter (regression coefficient) indicates the strength of how fertilization shifts the abundance of each species (positive slopes indicate an increase in abundance). The regression coefficients were termed 'effect size' (raw/relative abundance). We also calculated the community mean species effect size as the sum of each plant species' effect size (relative abundance) ÷ the number of plant species in each community. Hence, communities with negative/positive values of this index would correspond to communities that include more diluter/amplifier species.

To test the linear relationships between the amounts of nitrogen added and the various community-level indices (host species richness, *p*, pathogen load, seven community-weighted mean functional traits, and community mean effect size), we set each community-level index as the response variable and the amount of nitrogen addition as the independent variable in a series of linear models. We set P_i as the response variable and species effect size (raw abundance) as the independent variable to test the linear correlation between them. We also set the community mean effect size (relative abundance) or the total number of individual plants in the community as response variables separately, and host species richness as the independent variable in linear models. We also plotted the total number of individual plants in the community relative to the different nitrogen-fertilization groups using boxplots. We calculated the information-theoretic Akaike's information criterion corrected for small sample sizes (AIC_c) to evaluate relative support for each linear model we built. We used the information-theoretic evidence ratio (ER, $wAIC_c$[slope model] : $wAIC_c$[intercept-only model]) as an index of relative support for the linear slope model versus the intercept-only (null) model; when ER > 1.5, we deemed that there was evidence to support the slope model [13,38]. ERs were derived from tests of the contrasting model (hypothesis) to the null, and hence in this way, there was no need to default to arbitrary *p*-value thresholds. This statistical approach explicitly accounts for multiple models by calculating relative model probabilities ($wAIC_c$), and any variable collinearity via a parameter-count penalty [38]. In addition to the statistical evidence (AIC weights, relative model probabilities, and ER), we also calculated the per cent deviance explained in the response variable (*De*) as an index of each model's goodness of fit (GIF) [13,38].

To identify the most parsimonious models (i.e. greatest explanatory power for the fewest number of predictors according to $wAIC_c$) between pathogen load and the predictors (community competence, species richness, evenness, and the amount of nitrogen added), we constructed a series of generalized linear models using the `glm` function in the R package `stats`. We calculated the Spearman rank-order correlation between indices we considered using the `cor.test` function. We validated the use of a gamma family for the modelled error distribution based on the normalized scores of standardized residual deviance. We also calculated AIC_c, ER, and *De* for each model. We also applied structural equation modelling (SEM) to the predicted causal relationships between aforementioned variables on pathogen load. We determined the SEM GIF with a χ^2 test, the GIF value, and root mean-squared error of approximation (RMSEA). We did all SEM analyses using AMOS 18.0 (IBM, Chicago, IL). We did all other statistical analyses using R v. 2.15.1 [39].

3. Results

(a) A functional-based mechanism drives species loss under fertilization

We observed a total of 63 species in the 48 main experimental plots in 2015 (electronic supplementary material, table S1). Species richness decreased with an increase in the amount

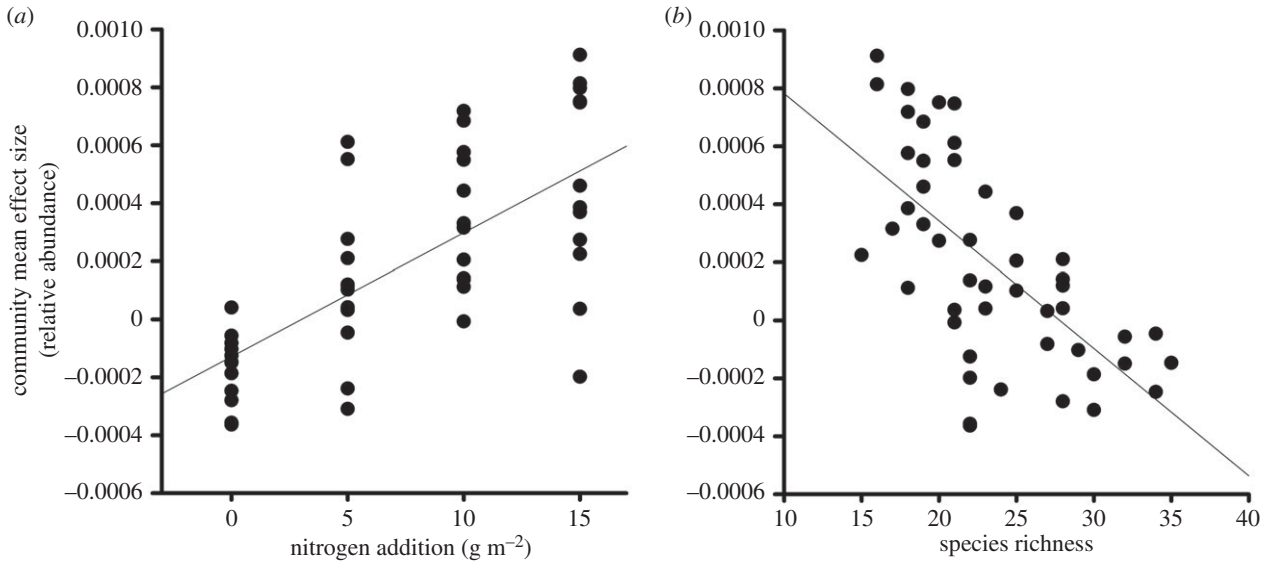

Figure 2. Linear relationship between host species richness, nitrogen addition, and community mean species effect size (sum of each plant species effect size [the regression coefficient of the linear slope of the relative abundance–nitrogen relationship] ÷ number of plant species in each community) of 48 main experimental plots in 2015 (there are some overlapping points). (*a*) Nitrogen addition (information-theoretic evidence ratio [ER] = 1.52×10^6; per cent deviance explained [*De*] = 47.30). (*b*) Host species richness (ER = 1.94×10^5; *De* = 42.58).

of nitrogen added, ranging from an average of 28.0 ± 1.4 species in control plots to 19.3 ± 0.8 species in plots receiving 15 g m^{-2} of nitrogen addition (i.e. a mean reduction of 31%; figure 1*a*). The average raw abundances of 12 of 63 species (19.0%) increased along the nitrogen-addition gradient; these species show some compensatory increases in abundance after nitrogen addition. Only two species (*Anemone rivularis* and *Festuca sinensis*, 3.2%) had relatively strong compensatory increases (species effect size [raw abundance] more than 3; electronic supplementary material table S1). The raw abundance of 46 species decreased (73.0%) along the nitrogen-addition gradient (including 12 species that were extirpated in the 15 g m^{-2} nitrogen-addition plots), the mean raw abundance of only one species (1.6%) remained unchanged after fertilization and four mean abundance relationships could not be tested (i.e. insufficient degrees of freedom; electronic supplementary material, table S1).

Among the seven community-weighted mean functional traits, average plant height (ER = 177.23), maximum plant height (ER = 23.91), leaf phosphorus content (ER = 19.99), and *per capita* seed mass (ER = 4.54) increased, whereas leaf nitrogen content (ER = 7.18×10^4) and the N–P ratio (ER = 1639.7) decreased along the nitrogen-addition gradient (table 1 and electronic supplementary material, figure S1). In other words, adding nitrogen fertilizer precipitated a change in species composition towards taller species that produced more seeds, with leaves that were richer in phosphorus, poorer in nitrogen and with lower N–P ratios. We therefore conclude that a functional-based mechanism underlies the fertilization-driven species loss.

(b) Strong community composition shifts under nitrogen fertilization

The total number of individual plants in the community in control plots (mean ± standard error (s.e.) = 207.58 ± 13.53) and in plots receiving 5 g m^{-2} of nitrogen addition (227.25 ± 16.23) were higher than in the 10 g m^{-2} (133.58 ± 11.16) and 15 g m^{-2} (114.67 ± 9.35) nitrogen-addition plots (electronic

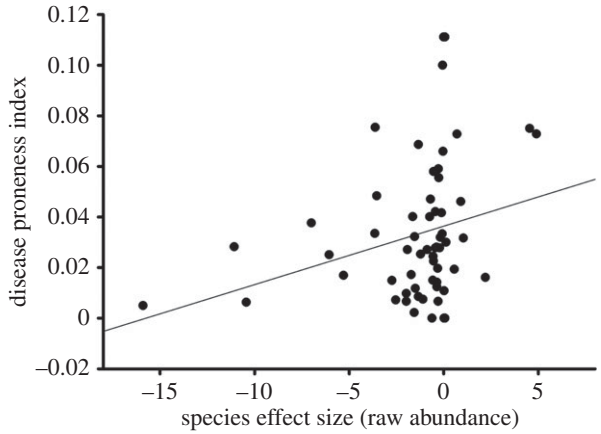

Figure 3. Disease proneness index (average proportion of leaf area of the specific plant species infected by disease, derived from the 32 natural plots) increased linearly with species effect size based on raw abundance (the regression coefficient of the linear slope of abundance–nitrogen relationship) in 2015 (information-theoretic evidence ratio [ER] = 3.66; per cent deviance explained [*De*] = 7.8), which means that the non- or poorly competent host species are most at risk of loss under fertilization.

supplementary material, figure S2). On the other hand, we found strong evidence for a positive correlation between total number of individual plants in the community and plant species richness (ER = 1.61×10^4; *De* = 42.13), which indicated little evidence for compensatory increases in abundance with decreased diversity following fertilization (electronic supplementary material, figure S3).

We found strong evidence for a positive correlation between nitrogen addition and community effect size based on relative abundance in our experimental plots (ER = 1.52×10^6; *De* = 47.30; figure 2*a*), while also strong evidence for a negative correlation between plant species richness and community effect size (ER = 1.94×10^5; *De* = 42.58; figure 2*b*). This indicated that the plots with less nitrogen fertilization (especially for the 0 g m^{-2} nitrogen-addition plots), or higher plant species richness (especially for plots with plant species richness more than 30), contained a greater

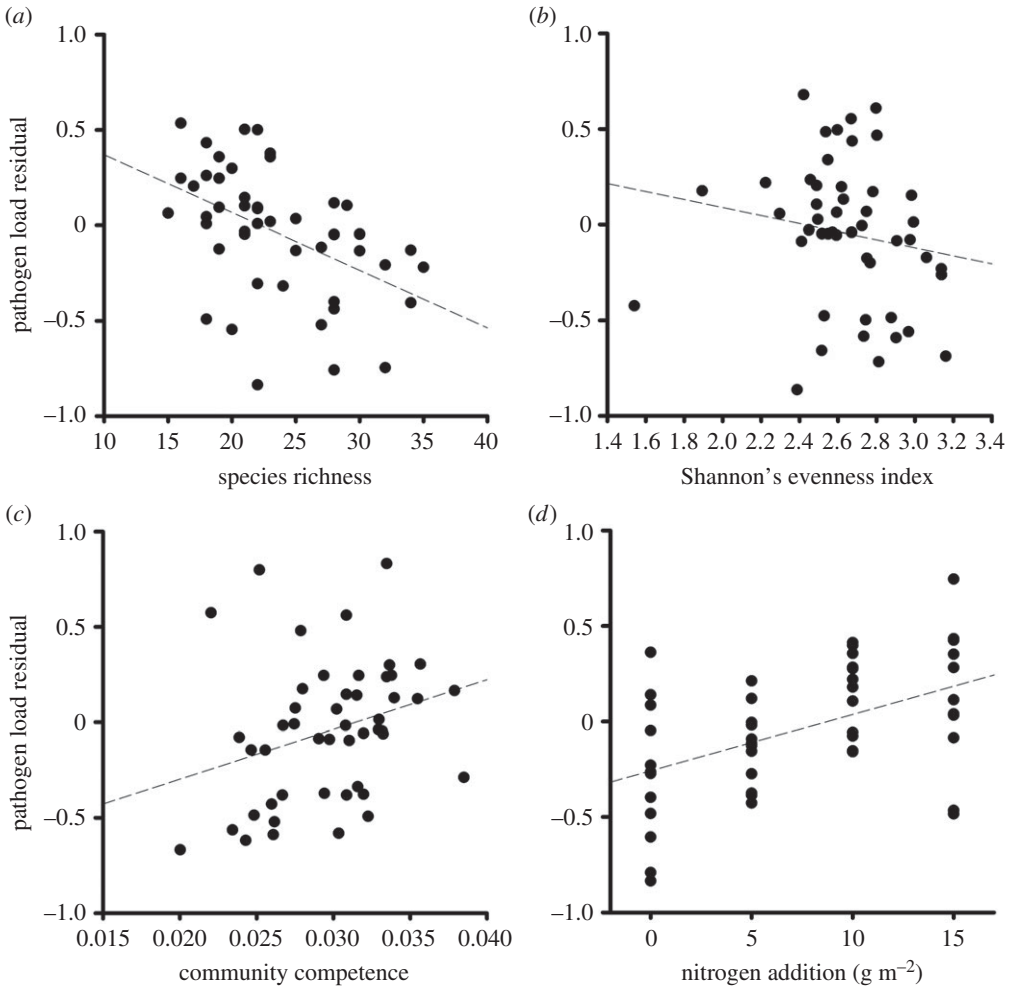

Figure 4. Partial residual plots of each single diversity index in the generalized linear models (boldface rows in table 2) to predict variation in pathogen load of 48 main experiment plots in 2015 (there are some overlapping points in this figure). (*a*) Host species richness (S_h), (*b*) Shannon's evenness index for host (H'_h), (*c*) community competence (p), and (*d*) nitrogen-fertilization treatment (N). Dashed lines are least-squares linear regression lines.

proportion of diluter species (community mean effect size based on relative abundance less than 0), showing strong community composition shifts under nitrogen fertilization even based on the relative abundance of plant species.

(c) Species effect size and community competence

The species effect size (raw abundance) varied from −15.90 to 4.90, while the P_i (the average proportion of leaf area of the specific plant species infected by disease) varied from 0 to 0.111 (e.g. 0–11.1%) for host plant species (electronic supplementary material, table S1). Species' P_i increased with this effect size (ER = 3.66; figure 3), meaning that species that increased in raw abundance in response to nitrogen addition were more prone to disease. At the community level, p increased along the nitrogen-addition gradient in 2015 (figure 1*b*), indicating an increase in the abundance of more competent species, and a high community capacity to support fungal diseases in fertilization plots.

Community pathogen load was negatively related to species richness (dilution effect), while it was positively related to nitrogen fertilization and p (figures 1*c* and 4). Among all generalized linear models constructed to explain variation in pathogen load (including the intercept-only model), the most parsimonious ($wAIC_c = 0.371$) included p and the amount of nitrogen addition, accounting for more than 35% of the deviance explained in pathogen load; species richness had little additional explanatory power in terms of deviance explained (table 2). However, the best single predictor of pathogen load was species richness ($AIC_c = -105.138$; $De = 24.4\%$), instead of p ($AIC_c = -103.672$; $De = 22.1\%$) or the amount of nitrogen added ($AIC_c = -96.182$; $De = 9.3\%$).

Given minor collinearity between some variables (electronic supplementary material, table S2), we applied SEMs to the same datasets. The final SEM ($\chi^2_2 = 1.941$, $p = 0.38$, GFI = 0.98, RMSEA = 0.0001, AIC = 27.94; standardized path coefficients are given in electronic supplementary material, figure S4) explained approximately 26.8% of the variance of pathogen load (electronic supplementary material, figure S4). This model revealed that host species richness (indirect effect) rather than nitrogen-fertilization treatment (direct effect) increased pathogen load. Standardized path coefficients of host species richness, Shannon's evenness index for host community competence, and nitrogen-fertilization treatment (direct effect) on pathogen load were −0.430, 0.171, 0.091, and 0.213, respectively (electronic supplementary material, figure S4).

4. Discussion

By integrating information on community structure, competence, and pathogen load in a 5-year nitrogen-addition experiment in an alpine meadow, our study provides strong empirical evidence of a functional link between fertilization and disease proneness of individual host species and entire

Table 2. Generalized linear model (error distribution family = gamma, link function = log) results for pathogen load (I) of 48 main experiment plots in 2015 as a function of host species richness (S_h), Shannon's evenness index for host (H'_h), community competence (p), and nitrogen-fertilization treatment (N). We included all possible combinations and the intercept-only (null) model. 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 per cent deviance explained (De) as a measure of the model's goodness of fit. Boldface rows highlight single-predictor models.

model	LL	k	AIC_c	ΔAIC_c	$wAIC_c$	De
$\sim N + p$	59.540	3	−110.150	0	0.371	35.1
$\sim S_h + N + p$	60.168	4	−108.907	1.243	0.199	36.7
$\sim H'_h + S_h + N + p$	60.914	5	−107.779	2.371	0.113	38.6
$\sim H'_h + N + p$	59.595	4	−107.762	2.388	0.112	35.2
$\sim S_h + p$	57.354	3	−105.778	4.372	0.042	29.0
$\sim H'_h + S_h + p$	58.556	4	−105.683	4.467	0.04	32.4
$\sim S_h + N$	57.100	3	−105.270	4.880	0.03	28.2
$\sim S_h$	**55.842**	**2**	**−105.138**	**5.012**	**0.03**	**24.4**
$\sim H'_h + S_h$	56.747	3	−104.563	5.587	0.02	27.2
$\sim H'_h + S_h + N$	57.716	4	−104.003	6.147	0.02	30.0
$\sim N$	**55.109**	**2**	**−103.672**	**6.478**	**0.01**	**22.1**
$\sim H'_h + N$	55.165	3	−101.400	8.750	<0.01	22.3
$\sim p$	**51.364**	**2**	**−96.182**	**13.968**	**<0.01**	**9.3**
$\sim H'_h + p$	51.889	3	−94.847	15.303	<0.01	11.2
~ 1	48.971	1	−93.676	16.474	<0.01	0.0
$\sim H'_h$	**49.953**	**2**	**−93.360**	**16.790**	**<0.01**	**3.9**

plant communities. Community competence for disease increased and the proportion of diluter species decreased along the nitrogen gradient, which suggests that enhanced biodiversity increases the dilution effect at the community level. At the same time, there was an indirect effect on disease severity of nitrogen addition via a change in community structure through the loss of less-competent species. By measuring a disease proneness index and other plant traits for each species in the control plots, and by employing both multiple working hypotheses and path-analysis approaches, we were able to separate the indirect and direct effects of fertilization on disease severity. Furthermore, both the amount of nitrogen added (direct effect) and community competence (indirect effect) played important roles in driving the community pathogen load under nitrogen fertilization.

(a) Predictable changes in host community structure under fertilization lead to the dilution effect

We attribute the dilution effect of reduced community pathogen load with increasing species richness under fertilization to: (i) the positive effect of nitrogen addition on the growth of fungal pathogens (direct effect; i.e. the nitrogen-disease hypothesis; [14,15]), (ii) the predictable changes in host community competence under fertilization, (iii) the compensatory increases in host abundance with decreasing host diversity, and, (iv) the physical isolation of non-host species [40–42]. For the specific pathogen–host system, we investigated, increasing host species richness is expected to increase the interception of spores by non-hosts (physical isolation), alter microclimatic conditions (e.g. temperature, humidity, illumination, raindrop splash) and increase spatial heterogeneity as well as the three-dimensional space-filling capacity (the complexity of vertical structure), which ultimately reduces pathogen load in species-rich assemblages [22,24]. Additionally, fertilization weakened the dilution effect in our experiment because of the increasing of community competence realized along the nitrogen-addition gradient [13].

A functional-based mechanism (total competition hypothesis) received more support from our experimental manipulation. The community-weighted mean average and maximum height increased while the community-weighted mean leaf nitrogen content decreased along the fertilization gradient, indicating that these were likely an outcome of a trade-off in competitive ability for light (above-ground) and nutrients (below-ground) [7,8]. The combination of above-ground (light) and below-ground competition (soil resources) therefore engendered competitive exclusion, an explanation consistent with several previous studies [5,19,43]. We also determined that the abundance of some leguminous forbs (*Oxytropis kansuensis*, *Tibetia himalaica*, and *Thermopsis lanceolala*) were especially vulnerable to declines after adding nitrogen (electronic supplementary material table S1), because these species typically had low average and maximum height and high leaf nitrogen. This probably results from the additive or interactive effects of root and shoot competition, rather than from light competition alone [5,27].

(b) Potential linkages between host competitive ability and disease proneness

The mechanism underlying the positive relationship between fertilization and host species proneness might also arise

because of a trade-off between specific defence systems and competitive ability [28,30]. Hence, low-proneness host species are the most at risk of extirpation under fertilization because they are relatively poor competitors, even in our specific pathogen–host system; in the control plots, these species would have likely contributed to the observed dilution effect [1]. Lacroix *et al*. [26] also found a negative relationship between competence and the order of species loss for generalist aphid-transmitted viral pathogens in natural assemblages, which is consistent with our conclusion even though we focused on relatively specialist fungal pathogens under nitrogen addition. One possible explanation for this negative relationship is the trade-off between resource investment in competition and defence in the context of resource limitation, and the higher relative cost to plants of defending themselves against specialist versus generalist enemies [44].

Studies in disease epidemiology support linkages between a host species' life-history traits and its competence for hosting disease [27,45–47]. Investment in resistance to infections can elicit a fitness cost, so species with a high resilience to disturbance can invest less in defence against (specialist) pathogens [48]. Even in our specific plant–fungal system, the non-random loss of species under fertilization increased the overall community's capacity to support fungal diseases. As human activity increases the availability of nutrients in terrestrial ecosystems through the addition of fertilizers [7,8], this action might additionally threaten ecosystem function by increasing disease risk.

Disease proneness, as well as the capacity of plant species to support diseases, might be exacerbated by increasing niche dimensionality as much as by the effect of light or resource availability [25]. Nitrogen fertilization can increase the disease pressure of plant species by both direct and indirect effects. In our relatively specific pathogen–host system, the reduction of plant species precipitates the loss of species and phylogenetic diversity of the remaining fungal pathogens, thus making plant species face similar pathogens and intensity of disease pressure under fertilization [49]. Such a process further promotes the loss of plant species via a reduction in the niche dimensions of fungal diseases.

Our results help to strengthen the functional foundation underlying the dilution effect by demonstrating that nitrogen fertilization can lead to a predictable relationship between the raw abundance and distribution and disease proneness of species in a community, in which low-diversity communities support a greater proportion of species with high proneness to disease [1,26], even in specific pathogen–host systems. Our results show the consistency of the 'ubiquity–competence' relationship in both specialist- and generalist-pathogen systems [26]. Grassland ecosystems, including alpine meadows of the Tibetan plateau, have undergone major changes in community structure because of past nitrogen deposition [8]. Hence, our results suggest that disease amplification in alpine meadows could have arisen from the transformation to highly disease-competent communities that were initially dominated by less competent species, which might be an underlying mechanism of the dilution effect. Our study therefore improves our knowledge at the interface of community structure and disease ecology to model pathogen transmission in complex plant–fungal systems, and provides general insights into the dynamics of infectious diseases under scenarios of increasing human interference of terrestrial ecosystems.

Data accessibility. The data supporting this article can be found in electronic supplementary material and Dryad Digital Repository [50].

Authors' contributions. S.Z. conceived and designed the study. X.L., S.L., and D.S. collected the data. X.L. and S.Z. performed the analyses. X.L., C.J.A.B., and S.Z. wrote the manuscript.

Competing interests. We declare we have no competing interests.

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References

1. Johnson PT, Preston DL, Hoverman JT, Richgels KL. 2013 Biodiversity decreases disease through predictable changes in host community competence. *Nature* **494**, 230–233. (doi:10.1038/nature11883)
2. Tilman D, Isbell F, Cowles JM. 2014 Biodiversity and ecosystem functioning. *Annu. Rev. Ecol. Syst*. **45**, 471–490. (doi:10.1146/annurev-ecolsys-120213-091917)
3. Isbell F, Reich PB, Tilman D, Hobbie SE, Polasky S, Binder S. 2013 Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proc. Natl Acad. Sci. USA* **110**, 11 911–11 916. (doi:10.1073/pnas.1310880110)
4. Veresoglou SD, Barto EK, Menexes G, Rillig MC. 2013 Fertilization affects severity of disease caused by fungal plant pathogens. *Plant Pathol*. **62**, 961–969. (doi:10.1111/ppa.12014)
5. Suding KN, Collins SL, Gough L, Clark C, Cleland EE, Gross KL, Milchunas DG, Pennings S. 2005 Functional-and abundance-based mechanisms explain diversity loss due to N fertilization. *Proc. Natl Acad. Sci. USA* **102**, 4387–4392. (doi:10.1073/pnas.0408648102)
6. Clark CM, Tilman D. 2008 Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature* **451**, 712–715. (doi:10.1038/nature06503)
7. Hautier Y, Niklaus PA, Hector A. 2009 Competition for light causes plant biodiversity loss after eutrophication. *Science* **324**, 636–638. (doi:10.1126/science.1169640)
8. Borer ET *et al.* 2014 Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* **508**, 517–520. (doi:10.1038/nature13144)
9. Seabloom EW *et al.* 2015 Plant species' origin predicts dominance and response to nutrient enrichment and herbivores in global grasslands. *Nat. Commun*. **6**, 7710. (doi:10.1038/ncomms8710)
10. Gough L, Osenberg CW, Gross KL, Collins SL. 2000 Fertilization effects on species density and primary productivity in herbaceous plant communities. *Oikos* **89**, 428–439. (doi:10.1034/j.1600-0706.2000.890302.x)
11. Hautier Y, Tilman D, Isbell F, Seabloom EW, Borer ET, Reich PB. 2015 Anthropogenic environmental changes affect ecosystem stability via biodiversity. *Science* **348**, 336–340. (doi:10.1126/science.aaa1788)
12. Lu M, Yang Y, Luo Y, Fang C, Zhou C, Chen J, Yang X, Li B. 2011 Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytol*. **189**, 1040–1050. (doi:10.1111/j.1469-8137.2010.03563.x)
13. Liu X, Lyu SM, Zhou SR, Bradshaw CJA. 2016 Warming and fertilization alter the dilution effect of host diversity on disease severity. *Ecology* **97**, 1680–1689. (doi:10.1890/15-1784.1)

14. Mitchell CE, Reich PB, Tilman D, Groth JV. 2003 Effects of elevated CO_2, nitrogen deposition, and decreased species diversity on foliar fungal plant disease. *Glob. Change Biol*. **9**, 438–451. (doi:10.1046/j.1365-2486.2003.00602.x)
15. Huber DM, Watson RD. 1974 Nitrogen form and plant disease. *Annu. Rev. Phytopathol*. **12**, 139–165. (doi:10.1146/annurev.py.12.090174.001035)
16. Williams DW, Powell AJ, Vincelli P, Dougherty CT. 1996 Dollar spot on bentgrass influenced by displacement of leaf surface moisture, nitrogen, and clipping removal. *Crop Sci*. **36**, 1304–1309. (doi:10.2135/cropsci1996.0011183X003600050039x)
17. Dawson W, Fischer M, Kleunen M. 2012 Common and rare plant species respond differently to fertilisation and competition, whether they are alien or native. *Ecol. Lett*. **15**, 873–880. (doi:10.1111/j.1461-0248.2012.01811.x)
18. Mitchell CE, Tilman D, Groth JV. 2002 Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. *Ecology* **83**, 1713–1726. (doi:10.1890/0012-9658(2002)083[1713:EOGPSD]2.0.CO;2)
19. Yang Z, Hautier Y, Borer ET, Zhang C, Du G. 2015 Abundance- and functional-based mechanisms of plant diversity loss with fertilization in the presence and absence of herbivores. *Oecologia* **179**, 261–270. (doi:10.1007/s00442-015-3313-7)
20. Burdon JJ, Chilvers GA. 1977 Controlled environment experiments on epidemic rates of barley mildew in different mixtures of barley and wheat. *Oecologia* **28**, 141–146. (doi:10.1007/BF00345249)
21. Knops JM *et al.* 1999 Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecol. Lett*. **2**, 286–293. (doi:10.1046/j.1461-0248.1999.00083.x)
22. Zhu YY *et al.* 2000 Genetic diversity and disease control in rice. *Nature* **406**, 718–722. (doi:10.1038/35021046)
23. Roscher C, Schumacher J, Foitzik O, Schulze ED. 2007 Resistance to rust fungi in *Lolium perenne* depends on within-species variation and performance of the host species in grasslands of different plant diversity. *Oecologia* **153**, 173–183. (doi:10.1007/s00442-007-0713-3)
24. Rottstock T, Joshi J, Kummer V, Fischer M. 2014 Higher plant diversity promotes higher diversity of fungal pathogens, while it decreases pathogen infection per plant. *Ecology* **95**, 1907–1917. (doi:10.1890/13-2317.1)
25. Harpole WS, Tilman D. 2007 Grassland species loss resulting from reduced niche dimension. *Nature* **446**, 791–793. (doi:10.1038/nature05684)
26. Lacroix C, Jolles A, Seabloom EW, Power AG, Mitchell CE, Borer ET. 2014 Non-random biodiversity loss underlies predictable increases in viral disease prevalence. *J. R. Soc. Interface* **11**, 20130947. (doi:10.1098/rsif.2013.0947)
27. Rajaniemi TK. 2002 Why does fertilization reduce plant species diversity? Testing three competition-based hypotheses. *J. Ecol*. **90**, 316–324. (doi:10.1046/j.1365-2745.2001.00662.x)
28. Huang ZY, de Boer WF, van Langevelde F, Olson V, Blackburn TM, Prins HH. 2013 Species' life-history traits explain interspecific variation in reservoir competence: a possible mechanism underlying the dilution effect. *PLoS ONE* **8**, e54341. (doi:10.1371/journal.pone.0054341)
29. Leibold MA. 1989 Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. *Am. Nat*. **134**, 922–949. (doi:10.1086/285022)
30. Viola DV, Mordecai EA, Jaramillo AG, Sistla SA, Albertson LK, Gosnell JS, Cardinale BJ, Levinec JM. 2010 Competition-defense tradeoffs and the maintenance of plant diversity. *Proc. Natl Acad. Sci. USA* **107**, 17 217–17 222. (doi:10.1073/pnas.1007745107)
31. Zhang R. 2009 *Master's thesis: survey and identification of the alpine grassland's major fungal diseases in Gannan region of Gansu province*. Lanzhou, China: Gansu Agricultural University.
32. Li J, Yang Y, Li B, Li W, Wang G, Knops JM. 2014 Effects of nitrogen and phosphorus fertilization on soil carbon fractions in alpine meadows on the Qinghai–Tibetan plateau. *PLoS ONE* **9**, e103266. (doi:10.1371/journal.pone.0103266)
33. Liu J, Zhang X, Song F, Zhou S, Cadotte M, Bradshaw CJA. 2015 Explaining maximum variation in productivity requires phylogenetic diversity and single functional traits. *Ecology* **96**, 176–183. (doi:10.1890/14-1034.1)
34. Hantsch L, Braun U, Scherer-Lorenzen M, Bruelheide H. 2013 Species richness and species identity effects on occurrence of foliar fungal pathogens in a tree diversity experiment. *Ecosphere* **4**, part81. (doi:10.1890/ES13-00103.1)
35. Hantsch L, Braun U, Haase J, Purschke O, Scherer-Lorenzen M, Bruelheide H. 2014 No plant functional diversity effects on foliar fungal pathogens in experimental tree communities. *Fung. Divers*. **66**, 139–151. (doi:10.1007/s13225-013-0273-2)
36. Flynn DFB, Mirotchnick N, Jain M, Palmer MI, Naeem S. 2011 Functional and phylogenetic diversity as predictors of biodiversity-ecosystem-function relationships. *Ecology* **92**, 1573–1581. (doi:10.1890/10-1245.1)
37. Oksanen J *et al.* 2013 *vegan: community ecology package*. R package version 2.0-7. See https://cran.r-project.org/web/packages/vegan/.
38. Burnham KP, Anderson DR, Huyvaert KP. 2011 AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behav. Ecol. Sociobiol*. **65**, 23–35. (doi:10.1007/s00265-010-1029-6)
39. R Development Core Team. 2014 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
40. Keesing F, Holt RD, Ostfeld RS. 2006 Effects of species diversity on disease risk. *Ecol. Lett*. **9**, 485–498. (doi:10.1111/j.1461-0248.2006.00885.x)
41. Ostfeld RS, Keesing F. 2012 Effects of host diversity on infectious disease. *Annu. Rev. Ecol. Syst*. **43**, 157–182. (doi:10.1146/annurev-ecolsys-102710-145022)
42. Johnson PT, Ostfeld RS, Keesing F. 2015 Frontiers in research on biodiversity and disease. *Ecol. Lett*. **18**, 1119–1133. (doi:10.1111/ele.12479)
43. Grime JP. 1973 Competitive exclusion in herbaceous vegetation. *Nature* **242**, 344–347. (doi:10.1038/242344a0)
44. Joshi J, Vrieling K. 2005 The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. *Ecol. Lett*. **8**, 704–714. (doi:10.1111/j.1461-0248.2005.00769.x)
45. Martin LII, Hasselquist D, Wikelski M. 2006 Investment in immune defense is linked to pace of life in house sparrows. *Oecologia* **147**, 565–575. (doi:10.1007/s00442-005-0314-y)
46. Lee KA, Wikelski M, Robinson WD, Robinson TR, Klasing KC. 2008 Constitutive immune defences correlate with life-history variables in tropical birds. *J. Anim. Ecol*. **77**, 356–363. (doi:10.1111/j.1365-2656.2007.01347.x)
47. Cronin JP, Welsh ME, Dekkers MG, Abercrombie ST, Mitchell CE. 2010 Host physiological phenotype explains pathogen reservoir potential. *Ecol. Lett*. **13**, 1221–1232. (doi:10.1111/j.1461-0248.2010.01513.x)
48. Miller MR, White A, Boots M. 2009 Host life span and the evolution of resistance characteristics. *Evolution* **61**, 2–14. (doi:10.1111/j.1558-5646.2007.00001.x)
49. Parker IM, Saunders M, Bontrager M, Weitz AP, Hendricks R, Magarey R, Suiter K, Gilbert GS. 2015 Phylogenetic structure and host abundance drive disease pressure in communities. *Nature* **520**, 542–544 (doi:10.1038/nature14372)
50. Liu X, Lyu SM, Sun DX, Zhou SR, Bradshaw CJA. 2016 Data from: Species decline under nitrogen fertilization increases community-level competence of fungal diseases. Dryad Digital Repository (doi:10.5061/dryad.r2s4n)