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Viral diversity and prevalence gradients in North American Pacific Coast grasslands

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Abstract. Host–pathogen interactions may be governed by the number of pathogens coexisting within an individual host (i.e., coinfection) and among different hosts, although most sampling in natural systems focuses on the prevalence of single pathogens and/or single hosts. We measured the prevalence of four barley and cereal yellow dwarf viruses (B/CYDVs) in three grass species at 26 natural grasslands along a 2000-km latitudinal gradient in the western United States and Canada. B/CYDVs are aphid-vectored RNA viruses that cause one of the most prevalent of all plant diseases worldwide. Pathogen prevalence and coinfection were uncorrelated, suggesting that different forces likely drive them. Coinfection, the number of viruses in a single infected host (α diversity), did not differ among host species but increased roughly twofold across our latitudinal transect. This increase in coinfection corresponded with a decline in among-host pathogen turnover (β diversity), suggesting that B/CYDVs in northern populations experience less transmission limitation than in southern populations. In contrast to pathogen diversity, pathogen prevalence was a function of host identity as well as biotic and abiotic environmental conditions. Prevalence declined with precipitation and increased with soil nitrate concentration, an important limiting nutrient for hosts and vectors of B/CYDVs. This work demonstrates the need for further studies of processes governing coinfection, and the utility of applying theory developed to explain diversity in communities of free-living organisms to pathogen systems.

Key words: alpha diversity; aphid-vectored RNA virus; barley and cereal yellow dwarf virus (B/CYDV); beta diversity; community ecology; disease ecology; Pacific coast, North America, grasslands.

Introduction

Host–pathogen interactions in natural systems take place within the context of complex food webs comprised of interacting hosts, pathogens, consumers, and vectors (Packer et al. 2003, Lello et al. 2004, Collinge and Ray 2006*a*, Cumming and Guegan 2006, Keesing et al. 2006, Borer et al. 2009*b*, Seabloom et al. 2009). Furthermore, the abiotic environment may ultimately mediate these community interactions (Guernier et al. 2004, Pope et al. 2005, Collinge and Ray 2006*a*, Cumming and Guegan 2006, Minakawa et al. 2006). For example, increased nutrient availability can increase vector populations of important human pathogens (such as malaria: Pope et al. 2005) and plant pathogens (such as barley and cereal yellow dwarf viruses; Borer et al. 2009*a*).

Despite the clear importance of community and environmental context, the community ecology of disease is a nascent field relative to the vast and well-developed literature on single host–pathogen interactions (Anderson and May 1986, Begon et al. 1992, Norman et al. 1994, Woolhouse et al. 2001, Holt et al. 2003, Dobson 2004). As a result of this focus on the dynamics of single pathogens, we have a greater understanding of the processes regulating pathogen prevalence than those that govern pathogen diversity. However, most hosts are coinfected by multiple pathogens, and coinfection (i.e., the number of pathogens coexisting within a host individual) can dramatically change pathogen virulence (i.e., the severity of the symptoms arising from infection) and transmission (Lello et al. 2004, Pedersen and Fenton 2007, Jolles et al. 2008, Seabloom et al. 2009).

Coinfection can increase host mortality in both plant and animal hosts (Lal et al. 1994, Miller and Rasochova 1997, Kamal et al. 2001*a*, *b*, Hood 2003, Tirado and Yoon 2003). For example, disease symptoms are much more severe for humans coinfected with malaria and human immunodeficiency virus, or the hepatitis C virus and the trematode *Schistosoma mansoni* (Lal et al. 1994, Kamal et al. 2001*a*, *b*, Tirado and Yoon 2003). Coinfection can alter transmission rates, reducing transmission by reducing vector efficiency, viral titers, or sporulation rates (Gildow and Rochow 1980, Gray et al. 1991, Wen et al. 1991, Power 1996, Al-Naimi et al. 2005). Conversely, coinfection can increase transmission by raising pathogen reproduction (as in antibody-

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dependent enhancement), lowering the ability of hosts to resist subsequent infections (e.g., immunodeficiency syndromes such as AIDS), or increasing the suite of efficient vectors (Rochow 1970*b*, Creamer and Falk 1990, Montefiori et al. 1996, Bentwich et al. 1999, Yazdanbakhsh et al. 2002, Coico et al. 2003, Tirado and Yoon 2003, Koskella et al. 2006, Jolles et al. 2008).

Given that coinfection can alter pathogen transmission rates and virulence, there is a clear need for studies that simultaneously investigate the regulators of both pathogen prevalence and coinfection. Coinfection can be regulated by factors operating at a wide range of spatial scales ranging from interactions taking place within single individuals, such as coinfection-induced changes in transmission and host mortality, to large-scale gradients in the climate, vector community, or host community (Dempster and Holmes 1995, Power 1996, Cumming and Guegan 2006, Borer et al. 2009*b*, Seabloom et al. 2009).

While similarly complex processes govern the diversity of free-living organisms, community ecology theory has been successful at describing emergent diversity patterns with fairly simple models. For example a basic organizing principle of community ecology predicts that local-scale diversity (α diversity) will reflect both the size of the species pool in the larger landscape (γ diversity) and the turnover in community composition among different sites (β diversity; Whittaker 1960, Caley and Schluter 1997, Whittaker et al. 2001). Simple mathematical relationships have been described for these community characteristics; the relationship among these diversity scales can be expressed as $\alpha = \gamma/\beta$ and $\beta = \gamma/\alpha$ (Whittaker 1960, Harrison et al. 1992) or as $\alpha = \gamma - \beta$ and $\beta = \gamma - \alpha$ (Whittaker et al. 2001, Dove and Cribb 2006). Recently, it has been suggested that the relationship between α, β, and γ diversity, that is well-studied in free-living organisms, can clarify the mechanisms governing coinfection by pathogens and parasites; however, empirical tests are lacking (Dove and Cribb 2006).

Dove and Cribb (2006) observe that coinfection (infracommunity diversity or within-host pathogen diversity) and α diversity are analogous, as are the total number of pathogens among coexisting hosts (component-community diversity) and γ diversity. In parasite communities with low β diversity (termed niche-assembled, interactive, or α-dominated communities; Holmes and Price 1986, Dove and Cribb 2006, Chase 2007), each host contains a similar and nearly complete set of the total pathogen pool. In these low β diversity communities, each pathogen is found in most hosts, implying that either pathogen transmission rates are high, or pathogen loss rates to recovery and mortality are low. As a result, each host has a predictable suite of pathogens and the within-host pathogen community is primarily governed by processes that determine pathogen diversity at larger spatial scales (i.e., γ diversity), such as a gradient in the physical environment or vector diversity (Guernier et al. 2004, Cumming and Guegan 2006, Dove and Cribb 2006, Chase 2007). In contrast, in communities with high β diversity (termed neutral, dispersal-assembled, isolationist, or β-dominated communities; Holmes and Price 1986, Dove and Cribb 2006, Chase 2007), individual hosts contain a small, random subset of the total pathogen species pool at any single site. In these communities with high β diversity, each pathogen is found in only a small proportion of hosts, implying that either transmission rates are low or pathogen loss rates are high. Furthermore, within-host pathogen communities in high β diversity systems are largely driven by stochastic infection events, and the pathogen community within any given host is largely unpredictable (Dove and Cribb 2006).

As with coinfection, pathogen prevalence may be governed by processes acting at a broad range of spatial scales. At larger scales, spatial or temporal variability in the abiotic environment can directly affect transmission of different pathogens by altering vector reproduction, vector community composition, pathogen reproduction, and pathogen transmission (Gregory 1973, Agrios 1978, Fitt et al. 1989, Madden et al. 1996, Aylor 1999, Cumming and Guegan 2006). For example, rainfall, temperature, and nutrient supplies have been shown to alter the prevalence of a range of diseases (Townsend et al. 2003, Zhou et al. 2004, Pope et al. 2005, Minakawa et al. 2006, Snall et al. 2008, Borer et al. 2009*a*). Recently, abiotic effects on both vectored and directly transmitted pathogens have received a great deal of attention within the context of human alteration of global climate and nutrient supply rates (Lindsay and Birley 1996, Harvell et al. 1999, Reiter 2001, Harvell et al. 2002, Townsend et al. 2003, Pounds et al. 2006, Dobson 2009).

As with abiotic gradients, large-scale gradients in host community composition can regulate generalist pathogens via a variety of pathways. In particular, hosts in communities dominated by highly competent hosts (i.e., hosts that readily carry and transmit a pathogen) can experience elevated transmission rates, infection risk, and overall pathogen prevalence (Keesing et al. 2006). For vectored pathogens, the composition of host communities can control the encounter rates between vectors and highly competent hosts (e.g., "dilution" or "spillover"; Power and Mitchell 2004, Keesing et al. 2006). Host composition also can control vector abundance when vector population dynamics depend upon host composition (Keesing et al. 2006, Borer et al. 2009*a*, Dobson 2009).

Coinfection may also arise from host-specific interactions with the pathogen and/or vector. For example, hosts may differ in their innate susceptibility to infection (Macdonald 1957, Service 1976, Ault 1994, Shrestha et al. 2006), desirability or value to vectors (Malmstrom et al. 2005*b*, Borer et al. 2009*a*), or their transmission competence and role as a reservoir species for the pathogen (LoGiudice et al. 2003, Power and Mitchell

2004). Thus, at a single location different host species may exhibit consistent differences in coinfection.

Thus, while it is widely acknowledged that host and pathogen community context mediate the distribution of pathogens among hosts in natural systems, few large-scale studies simultaneously investigate the effects of the abiotic and biotic environment on a community of pathogens in multiple hosts. To bridge this gap we surveyed the prevalence and diversity of four barley and cereal yellow dwarf viruses (B/CYDVs) in three host species in 26 natural grasslands along a 2000 km latitudinal gradient in the western United States and Canada (Fig. 1). We measured infection by four B/CYDV pathogens in three hosts from three different tribes in the subfamily Poodieae that differ strongly in their interactions with both pathogens and their aphid vectors: *Avena fatua* (an exotic annual that is highly valuable to vectors and strongly impacted by the pathogen at the individual level), *Bromus hordeaceus* (an exotic annual that is highly valuable to vectors and strongly impacted by the pathogen at the individual level), and *Elymus glaucus* (a native perennial that is of low value to vectors but strongly impacted by the pathogen at the individual level; see Appendix: Table 1; and the *Study system* section). We also tested site-level abiotic and biotic factors that have been suggested as drivers of pathogen dynamics, including rainfall, soil nitrogen, and host and non-host biomass and abundance.

We use these data to investigate the effects of the abiotic environment, host community composition, and host identity on prevalence and coinfection. We also use the changes in pathogen α, β, and γ diversity to determine the relative importance of niche and dispersal assembly in the pathogen community along this large geographic gradient. Specifically, we address the following four questions. (1) Does host identity alter pathogen prevalence or diversity? (2) Does the abiotic environment (latitude, precipitation, or soil resources) alter pathogen prevalence or diversity? (3) Does the host community (plant standing crop, host richness, and relative abundance of non-hosts, low-quality hosts, and high-quality hosts) alter pathogen prevalence or diversity? (4) Is coinfection regulated by the total pathogen pool at a site (i.e., low β diversity or niche-assembled communities) or the turnover in pathogens among coexisting hosts (i.e., high β diversity or neutral-assembled communities)?

In addition to providing insight into this important suite of grassland pathogens, a further goal of this work is to demonstrate the feasibility of using observational data to investigate the biotic and abiotic drivers of pathogen diversity across spatial scales that extend beyond those that are feasible for experimentation.

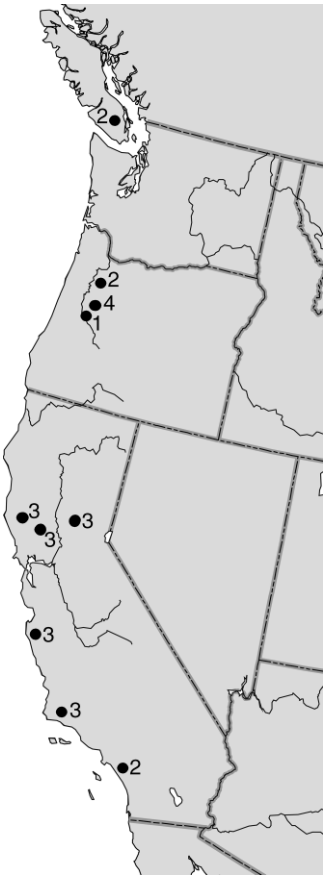

FIG. 1. Study sites (solid circles) for field survey of barley and cereal yellow dwarf viruses (B/CYDV) prevalence and diversity on the Pacific coast of North America (USA and Canada). Where symbols for multiple populations overlap, numbers indicate the number of populations sampled.

STUDY SYSTEM

B/CYDVs are a suite of aphid-vectored RNA viruses in the Luteoviridae known to infect over 150 grass species in over 40 different genera (Irwin and Thresh 1990, D'Arcy 1995). These known hosts are distributed widely throughout the Poaceae and include five of the six subfamilies and 11 of the 25 tribes (Irwin and Thresh 1990, D'Arcy 1995). These phloem-limited pathogens cause one of the most devastating diseases of small grain crops and are some of the most prevalent of all viral pathogens globally (Irwin and Thresh 1990). Recent work suggests that the presence of these viruses was likely a necessary precondition to one of the most widespread and persistent plant invasions worldwide (Malmstrom et al. 2005*b*, Borer et al. 2007), the conversion of 25% of the area of California to annual grassland dominated by exotic species from the Mediterranean region (Heady 1977, Seabloom et al. 2003).

Infection by B/CYDV leads to increased mortality, stunted growth, and decreased fecundity in crops and grasses (Rochow 1970*a*, D'Arcy 1995, Malmstrom et al. 2005*a*). There is no vertical transmission of the viruses; all seedlings of infected parents are initially uninfected (Rochow 1970*a*). The lack of vertical transmission means that the pathogen cannot persist in a strictly

annual system with non-overlapping generations (i.e., strong seasonality). In these systems, epidemics are likely to be started by the arrival of viruliferous, migrant aphids from distant areas where they persist year round (Irwin and Thresh 1988, Hewings and Eastman 1995). The presence year round of irrigated cereal crop production can allow local persistence in annual-only systems, and has been associated with increased pathogen prevalence in crops (Hewings and Eastman 1995). These viruses also can persist in the vegetative parts of wild perennial grasses (Borer et al. 2007).

B/CYDVs are transmitted by at least 25 different aphid species worldwide (Halbert and Voegtlin 1995). The viruses do not replicate in the aphid vectors and are not transmitted to aphid offspring (Rochow 1970*a*, Agrios 1978). Aphids can acquire the viruses in as short as 15 minutes, and viruliferous aphids can inoculate a plant in two hours, though efficiency increases with acquisition and inoculation time (Gray et al. 1991, Power et al. 1991).

BYDV and CYDV species belong to distinct genera, and interactions among the different viral species can alter the transmission and virulence of B/CYDVs (Miller and Rasochova 1997). For example, cross protection occurs between BYDVs but not between BYDVs and CYDVs (Miller and Rasochova 1997). While congeneric viruses can provide a degree of cross protection, infection by viruses from different genera (e.g., BYDV-PAV and CYDV-RPV) can lead to increased disease severity (Miller and Rasochova 1997). In general, cross protection and synergistic mortality from mixed infection should lead to lower levels of coinfection, although this has not been detected in field populations (Seabloom et al. 2009).

Host species differ strongly in their interactions with these viral pathogens and their vectors (Appendix: Table 1). In the broadest sense, aphid vectors have two to three times higher colonization and fecundity on annual hosts than on perennial hosts, leading to higher observed host-prevalence rates in the field (Malmstrom et al. 2005*b*, Borer et al. 2007, 2009*a*). Among annual grasses, *A. fatua* (one of our target hosts) is a particularly competent host for B/CYDV pathogens, creating a strong spillover effect on adjacent host species (Power and Mitchell 2004, Malmstrom et al. 2005*b*). In addition, pathogen prevalence and diversity in annual hosts represent the spread of B/CYDV pathogens within a single season, because all annual seedlings are uninfected at germination (i.e., our target annual hosts, *B. hordeaceus* and *A. fatua*). In contrast, perennial grass hosts (e.g., our target host, *E. glaucus*) do not generally lose infections among years but rather tend to accumulate B/CYDV pathogens throughout their lifetime, leading to an increase in prevalence over time (Dempster and Holmes 1995, Power and Mitchell 2004, Seabloom et al. 2009).

B/CYDV aphid vector species differ strongly in their efficiency at transmitting different viruses. The most common vectors at our study sites are *Rhopalosiphum padi*, *R. maidis*, *Sitobion avenae* (see Plate 1), *Metopolophium dirhodum*, and *Schizaphis graminum* (E. T. Borer, *unpublished data*). Of these species, *R. maidis* and *S. graminum* each efficiently transmit only a single viral species (BYDV-RMV and BYDV-SGV, respectively). In contrast, *R. padi* is an efficient vector for both BYDV-PAV and CYDV-RPV. Similarly, *S. avenae* and *M. dirhodum* efficiently transmit two BYDV species (BYDV-PAV and BYDV-MAV; Power and Gray 1995, Miller and Rasochova 1997, Leclercq-Le Quillec et al. 2000). Previous observations in West Coast grasslands, performed at smaller spatial scales than the current study, suggest that B/CYDV viruses are highly aggregated, and that their covariance structure among sites, years, and within hosts is strongly associated with their vector affinity, such that viral species that share a vector are positively correlated both spatially and temporally (Seabloom et al. 2009).

Methods

Field survey of pathogen prevalence and host community

We sampled B/CYDV prevalence in natural grasslands at 11 reserves in California, Oregon, and British Columbia (Fig. 1; Appendix: Table 2). At the larger reserves, we sampled up to three populations separated by at least 500 m (and usually much more) for a total of 26 sample sites. At each site, we collected at least 20 individuals each of *Bromus hordeaceus*, *Elymus glaucus*, and *Avena fatua* (979 individual hosts assayed). Note that not all hosts were present at all sites. We were not able to collect *A. fatua* at Hopland, Hoskins, and Horserock reserves, and we were not able to collect *B. hordeaceus* at Horserock Reserve; however, all results presented are qualitatively similar if we only examine sites at which we collected multiple host species. Hosts were collected at a similar phenological point at each site, the latest possible date at which we could still collect sufficient green tissue from the annual species. Peak aphid abundance generally occurs much earlier in the season (E. T. Borer, *unpublished data*), so this late season collection represents a point at which most of the transmission has occurred for the current season.

Host tissue samples were shipped by overnight mail for viral assays. Leaf tissue from each host was tested for infection by BYDV-PAV, BYDV-MAV, BYDV-SGV, and CYDV-RPV using enzyme-linked immunosorbent assay (ELISA; Rochow 1986) using antibodies from Agdia, Elkhart, Indiana, USA. To be conservative, in uncommon cases where putative infections by two serologically related viruses were associated nearly 1:1 within individuals of a host species at a site, we regarded the putative infections of the virus that elicited the weaker assay response (relative to standard controls on each microplate) as cross reactions to the other virus, rather than as coinfections. This left our estimate of prevalence unchanged, but somewhat reduced our estimate of coinfection.

At each site, we quantified plant biomass by clipping, drying to constant mass at 60°C, and weighing to the nearest 0.01 g all aboveground biomass from two 0.1 m × 1 m quadrats. We estimated the area covered by each plant species present in two 0.5 m × 1 m quadrats. Cover was estimated independently for each species, so total cover can sum to more than 100% in communities with multilayered canopies. We collected and air-dried three 2.5 cm × 10 cm deep soil cores that were analyzed for total phosphorous, nitrate, potassium, organic matter, sand, silt, clay, and pH by A & L Western Agricultural Laboratories (Modesto, California, USA).

Statistical analyses

All statistical analyses were conducted using R version 2.5.1 (R Development Core Team 2007). Prevalence data were analyzed with logistic regression, and diversity measures (viral richness within hosts or sites) were analyzed using Poisson regression. In both cases, we used a quasi-likelihood approach (quasibinomial and quasipoisson distributions in the glm function), because the data dispersion did not match the distributional assumptions of the binomial or Poisson distributions (McCullagh and Nelder 1989, Venables and Ripley 2003). In all cases, we tested for host differences and host × environment interactions by including host identity in the regression models. As there were few strong host × environment interactions, we then used a simpler regression model based on mean prevalence and diversity averaged across all hosts.

We tested whether viruses carried by the same vector were more highly correlated than expected at random using Mantel tests. Mantel tests are permutation tests that can be used to test whether multivariate distances in a distance matrix are greater among categories in a design matrix than those within categories, a conceptually similar analysis to a multivariate, nonparametric ANOVA (Mantel 1967, Manly 1986, Sokal and Rohlf 1995). In our analyses, the design matrix was constructed by assigning a value of one to elements representing viral species pairs that shared a vector and a zero to elements representing viral species pairs that did not share a vector (Sokal and Rohlf 1995, Seabloom et al. 2009). Significantly large positive values of the test statistic Z indicate that the viral community is more similar within than among vector affinity categories. The observed value of Z was compared to the distribution of values obtained in 1000 random permutations of the viral species correlation matrix. Mantel tests were performed using the mantel.test function in the ape R library.

Results

Our sample sites spanned a wide range of environmental conditions. Our collection locations spanned 15 degrees of latitude, from 33.8° to 48.8° N. Among these sites, rainfall during the 2006 growing season (August 2005 to July 2006) ranged from 224 mm to 1504 mm, and total standing crop ranged from 22 to 898 g/m^2. Soil chemistry was correspondingly variable among sites in nitrogen (2–10 ppm nitrate), phosphorus (4–47 ppm), potassium (55–485 ppm), pH (5–7.7), organic matter (2.1–6.9%), sand (34–74%), silt (12–48%), and clay (6–26%). Of these factors, only latitude, rainfall, and nitrate were significant factors in any model of prevalence or coinfection (Appendix: Tables 3, 4, and 5). We were missing soil nitrate data from three sites, so we conducted all regression analyses on the full data set without soil nitrate, and also on the more restricted data set that included the soil nitrate data. There was a strong positive relationship between prevalence and soil nitrate, so we separately present regression analyses on the full set of data without soil nitrate (Appendix: Tables 3, 4), and the univariate tests of soil nitrate effects on prevalence and coinfection (Appendix: Table 5).

The composition of the host community also varied widely across our 26 sampled populations. Non-host cover (i.e., cover summed across all forb species) ranged from 12% to 153% cover, low-quality host cover (i.e., cover summed across all perennial grass species) ranged from 0% to 68% cover, high-quality host cover (i.e., cover summed across all annual grass species) ranged from 0% to 130%, the percent of the community composed of hosts (total grass cover/total cover) ranged from 7–85%, and host (grass) species richness ranged from one to seven species per 0.5 m^2. Note that cover of each species was estimated independently, so summed cover values exceeding 100% indicate a multilayer canopy. The only host–community relationship in our regression models was a positive relationship between the cover of perennial grasses and prevalence in one host species (*B. hordeaceus*; Appendix: Table 3). We were missing plant-community biomass from six sites, so we repeated all regression analyses on the more restricted data set that included biomass. Community biomass was not significant in any models.

Prevalence of virus infection across all sampled hosts was 20.7%, and prevalence within sampled populations ranged from 0% to 70%. Single virus prevalence rates were as follows: 13.1% BYDV-PAV, 11.5% BYDV-MAV, 8.3% CYDV-RPV, and 3.6% BYDV-SGV. The mean number of viruses in infected hosts was 1.68. Prevalence and coinfection (the average number of strains in infected hosts) were uncorrelated among sites ($r = 0.06$). With 26 samples, the 95% confidence interval for r is $-0.35 < r < 0.45$ and accordingly r^2 could reach 0.12 or 0.20 given the variability in the estimate for r (i.e., the sign of r). Thus the vast majority (>88%) of the variability in coinfection remains unaccounted for by site-scale prevalence, suggesting that the primary drivers of prevalence and coinfection differ.

We tested whether the observed coinfection values were higher than expected by comparing them to the distribution of coinfection values calculated from 1000 random permutations of the individual viral species prevalence data. In our sample, coinfection was much

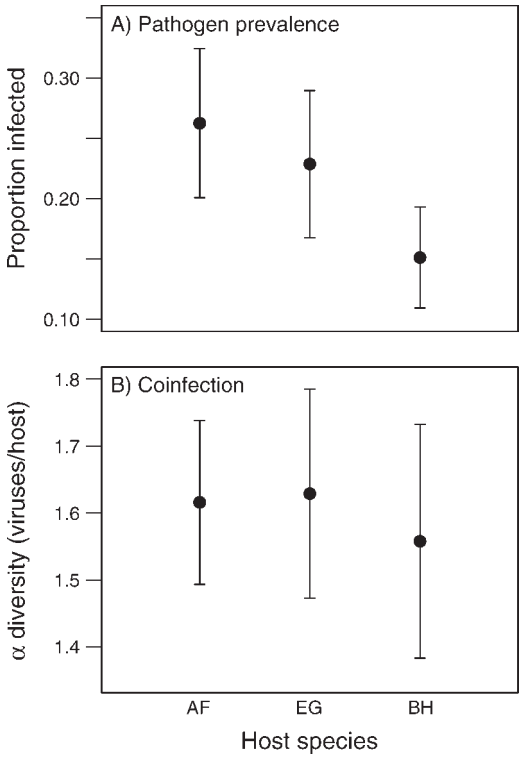

FIG. 2. (A) B/CYDV (barley and cereal yellow dwarf virus) prevalence and (B) viral α diversity or viruses per infected host for three host species: *Avena fatua* (AF), *Elymus glaucus* (EG), and *Bromus hordeaceus* (BH). *A. fatua* and *B. hordeaceus* are annual grasses; *E. glaucus* is a perennial bunchgrass.

higher than expected at random; the observed data had fewer singly infected and more multiply infected hosts than expected if the occurrences of the viruses were distributed randomly with respect to one another ($P < 0.001$). Of the infected hosts, 39% were infected with at least two viruses and 14% carried three or more viruses. By comparison, only 9% (range of 7–12%) of the hosts were expected to carry two viral species and <1% were expected to carry three species (range of 0–2%) in the permuted data.

Using a Mantel test, we found that viral species sharing a vector were significantly positively correlated ($P < 0.001$). For example, BYDV-PAV and CYDV-RPV, both carried by *R. padi*, had a correlation coefficient of 0.50 ($P < 0.0001$). Similarly, BYDV-PAV and BYDV-MAV, both vectored by *S. avenae* and *M. dirhodum*, had a correlation coefficient of 0.50 ($P < 0.0001$). In contrast, BYDV-SGV, the only B/CYDV viral species efficiently transmitted by *S. graminum*, had correlations very close to zero with all other viruses ($-0.04 < r < -0.02$). While BYDV-MAV and CYDV-RPV do not share a known vector, they were positively correlated ($r = 0.40$, $P < 0.001$), perhaps due to an indirect positive association with BYDV-PAV (BYDV-PAV and CYDV-RPV share a vector as do BYDV-PAV and BYDV-MAV).

Drivers of prevalence among sites

Pathogen prevalence differed among hosts, with *A. fatua*, an annual grass of high vector quality, and *E. glaucus*, a perennial bunchgrass of low vector quality, having higher prevalence than *B. hordeaceus*, an annual host of high vector quality (Fig. 2A). Prevalence declined strongly with precipitation and increased with soil nitrate (Fig. 3; Appendix: Tables 3, 4, and 5). The only significant interaction between host identity and any biotic and abiotic factors was with the cover of perennial grass (Appendix: Table 3). Pathogen prevalence increased in *B. hordeaceus* with increasing cover of perennial grasses, but was unchanged in *A. fatua* and *E. glaucus* (Appendix: Table 3).

Drivers of coinfection among sites

In contrast to site-level prevalence, coinfection (α diversity) did not differ among host species (Fig. 2B) and was correlated solely with latitude (Appendix: Table 3; Fig. 3). Coinfection increased from close to 1.0 at our southern sites to 2.5 at our northernmost sites (Fig. 3). This increase was not associated with any systematic change in the site-level pathogen diversity (γ diversity), which was virtually invariant over each gradient (Appendix: Table 3; Fig. 3). There was a strong decline in among-host viral diversity ($\beta = \gamma/\alpha$) with latitude, as expected, given the lack of variability in site-level pathogen diversity (Appendix: Table 3; Fig. 3). At our northern sites, β diversity was close to 1.0, because all infected hosts carried all available viral species ($\gamma \approx \alpha$). In contrast, at our southern sites, each host carried less than half the viral species found at the site ($\gamma \approx 2.5\alpha$; Fig. 3). We note that we find the same pattern of decreasing β diversity with latitude when we use the alternate formulation for β diversity ($\beta = \gamma - \alpha$), although the absolute values differ.

DISCUSSION

Spatial patterns of pathogen prevalence and diversity arise within the context of the abiotic environment and biotic pathogen, vector, and host communities; however, the importance of these factors is rarely assessed simultaneously. Here we tested the effects of host identity, abiotic environment (e.g., latitude, precipitation, or soil resources), and the host community (plant standing crop, host richness, and relative abundance of non-hosts, low-quality hosts, and high-quality hosts) on pathogen prevalence and diversity (see questions 1–3 in the *Introduction*). In addition, we tested whether changes in pathogen coinfection (α diversity) were primarily regulated by the total pathogen pool at a site (i.e., low β diversity or niche-assembled communities) or the turnover in pathogens among coexisting hosts (i.e., high β diversity or neutral-assembled communities; see question 4 in the *Introduction*).

We found that site-level pathogen prevalence and pathogen diversity within infected hosts were uncorre-

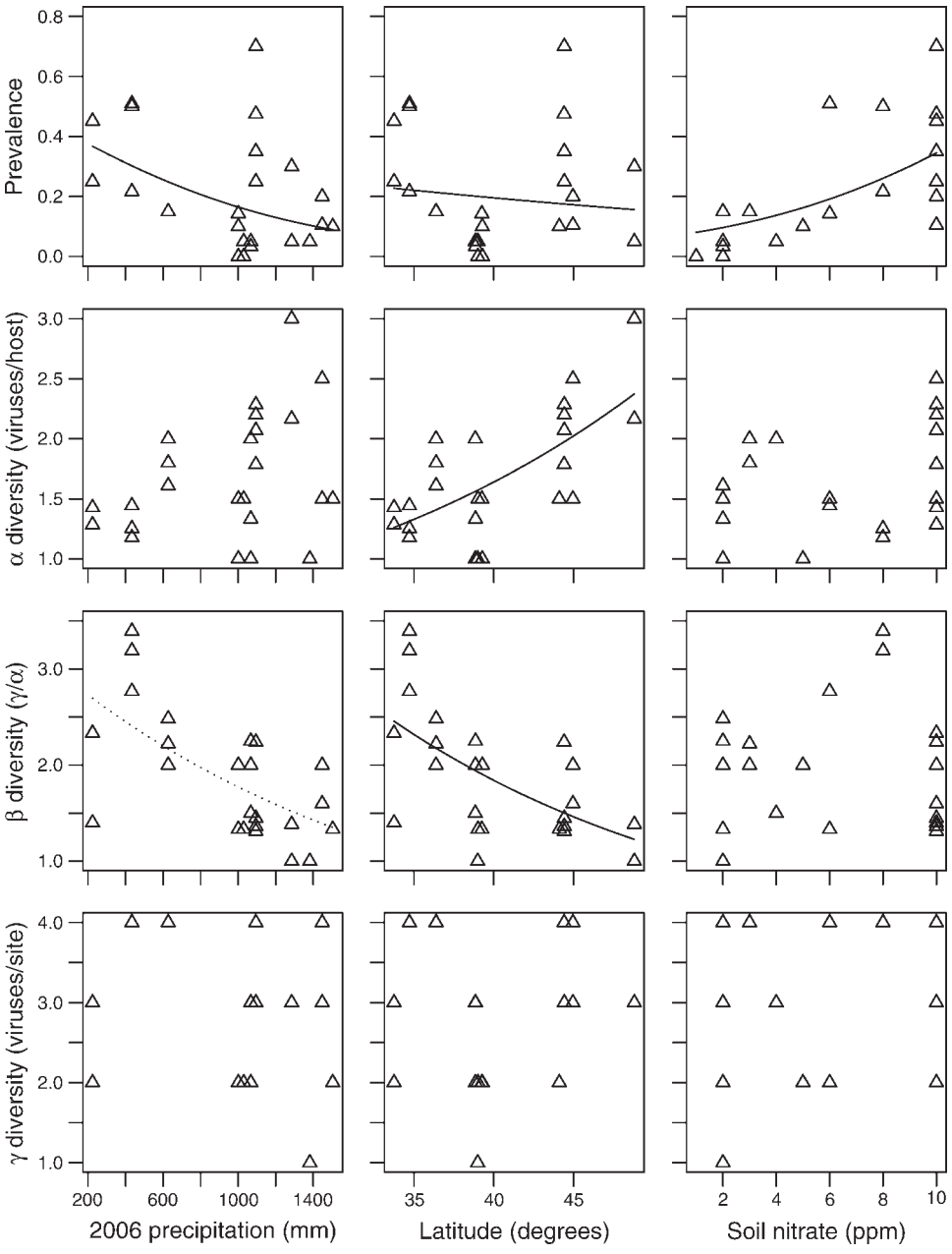

FIG. 3. Determinants of viral prevalence and viral species diversity (α, β, and γ) averaged across three grass hosts (*A. fatua*, *B. hordeaceus*, and *E. glaucus*) in grasslands on the West Coast of North America. Regression lines are univariate regressions and so differ from the slopes presented in the multiple regression models in the Appendix: Tables A3, A4, and A5. The dotted line indicates a regression that is significant in univariate regression but not in multiple regression models.

lated, suggesting that these patterns were generated by distinct processes. Site-level pathogen prevalence of this suite of vectored, generalist pathogens varied with host identity, while coinfection was similar among host species (question 1). Prevalence and coinfection were both correlated with gradients in the abiotic environment, although the driving factors differed (question 2). Infection prevalence decreased with precipitation and increased with soil nitrate. In contrast, coinfection in all host species increased with latitude but was independent of precipitation and nitrate. Recent theoretical work examining the processes relating prevalence and coinfection suggests that prevalence and coinfection differ in their response across a wide range of interactions among pathogens sharing a host (e.g., cross protection to synergistic mortality; Seabloom et al. 2009). Thus, theory supports the lack of relationship between prevalence and coinfection found here.

PLATE 1. *Sitobion avenae* (Fab.), the English grain aphid, is a globally distributed vector of two barley yellow dwarf viruses (MAV and PAV) and is commonly found throughout the geographic range of this study. Photo credit: E. T. Borer and Mikal' Davis.

The only effect of the host community context that we detected was an increase in prevalence in one host species (*B. hordeaceus*) with increasing perennial grass cover (question 3). It is unlikely that our failure to detect a general, direct effect of host community composition on viral prevalence or diversity was due to a lack of variability in our data. Across our sampled sites, total standing biomass varied 40-fold, host richness varied seven-fold, and the proportion of the community composed of hosts (grass cover/total cover) varied six-fold. However, experimental manipulation of host community composition may provide a more powerful approach for detecting the independent effects of the local host community composition, such as pathogen spillover (Power and Mitchell 2004, Malmstrom et al. 2005*b*).

The latitudinal gradient in coinfection was not driven by an increase in the total number of viral species present at our northern sites. Rather, infected hosts at northern sites carried a larger proportion of the available viral species pool, causing β diversity to decline with latitude (question 4 in the *Introduction*). This decline in β diversity is concordant with a switch from neutral communities with low transmission rates in the south to niche-assembled communities with high transmission at the northern sites (Dove and Cribb 2006). While higher rates of coinfection can, in general, arise not only from higher transmission rates, but also from lower loss rates of infection to recovery or mortality, this is less likely in our system because infected plants are not known to recover (Dempster and Holmes 1995). Furthermore, pathogen infections by the different B/CYD viruses were highly aggregated within hosts. B/CYD viruses efficiently transmitted by a shared vector were highly correlated across our sites, in concordance with an earlier study conducted using a single host collected across a much smaller spatial gradient (Seabloom et al. 2009). Theoretical work examining the drivers of coinfection suggests that the increase in coinfection with latitude may be driven by the local abundance of generalist vectors (Seabloom et al. 2009).

We selected a range of hosts for sampling that we expected a priori to differ strongly in their interactions with the B/CYD viruses and the aphid vectors. While prevalence rates differed strongly among hosts, the among-host differences we observed were not those we expected based on aphid preferences for annual hosts and high fecundity of aphids feeding on annual hosts (Malmstrom et al. 2005*b*, Borer et al. 2009*a*; Appendix: Table 1). Rather than finding higher prevalence in our annual hosts, we found that the strongest differences in prevalence were between the two annual hosts (26% infected for *A. fatua* and 15% infected for *B. hordeaceus*). We also found that the perennial host (23% infected for *E. glaucus*) had a prevalence similar to the highest annual host (*A. fatua*) and 53% higher than the annual host most preferred by aphids (*B. hordeaceus*; Appendix: Table 1). The higher-than-expected prevalence in the perennial host may reflect the more general patterns of gradual accumulation of viral infection over multiple years observed in other B/CYDV studies (Dempster and Holmes 1995, Power and Mitchell 2004, Seabloom et al. 2009).

The mismatch between prevalence and vector preference suggests that scaling population-level estimates of host–pathogen or host–vector interactions to larger spatial scales or complex natural communities may not be straightforward, and that the viral transmission properties of host species may not be consistent with their roles in vector regulation (Keesing et al. 2006). Future work to better characterize transmission competence and individual and population effects of infection on host species with a broad suite of characteristics will provide links to theory, allowing further insights into this field pattern, including the relative importance of host identity, host location, and host community context

in determining host infection risk (e.g., Keesing et al. 2006). Here, we have shown that both host composition and the environment, including precipitation, latitude, and soil nitrate, can be important predictors of infection prevalence.

Our results also support the importance of abiotic environmental context in disease dynamics. Here, pathogen prevalence rates increased with soil nitrate levels in all host species. Nutrients have been implicated in elevating infection prevalence and severity for focal hosts in a variety of recent terrestrial, freshwater, and marine studies (Bruno et al. 2003, Johnson et al. 2007, Walters and Bingham 2007). More specifically, nutrient supplies have been shown to mediate vector populations of plant and animal pathogens by changing host or habitat quality (Pope et al. 2005, Borer et al. 2009*a*). The potential for a general role of nutrient supplies on vectored pathogens is of particular importance given the current rate at which humans are adding nutrients to Earth's ecosystems (Vitousek et al. 1997, Tilman et al. 2001, Townsend et al. 2003).

We note here that ascribing causation based on observational data requires caution, because of the covariance among predictive variables. For example, we found that prevalence was highest at sites with low precipitation. However, precipitation and soil phosphorous were inversely correlated in our data set ($r = -0.54$), so these high prevalence sites also had higher soil phosphorous, an important limiting resource for some viruses (Clasen and Elser 2007). Ultimately, experimental manipulation of nutrients will be necessary to resolve the relative importance of abiotic factors that covary in these observational data.

As predicted by theory (Keesing et al. 2006), host community context also played a role in determining pathogen prevalence. However, this relationship was mediated by host–community interactions; prevalence in *B. hordeaceus* increased with the cover of perennial grasses, while prevalence in the other two focal hosts was unaffected of perennial grass cover. There is mounting evidence of the general importance of local host community composition and species richness in controlling pathogen prevalence. For example, increasing host richness is associated with reduced disease risk for several pathogens of both animals and plants (Mitchell et al. 2002, Ezenwa et al. 2006, Telfer et al. 2007), and compositional shifts that favor low competence rodent hosts can lower Lyme disease risk (LoGiudice et al. 2003). Our results emphasize that the effects of community context on disease risk may vary among focal host species. Thus, the characteristics of species in a community, and not simply total richness, may be critical to a more general understanding of host diversity effects on pathogen prevalence (Power and Mitchell 2004, Keesing et al. 2006, Mitchell and Power 2006).

While coinfection can have strong impacts on pathogen transmission and virulence and has been increasingly seen as an important risk factor in human diseases (Lal et al. 1994, Montefiori et al. 1996, Kamal et al. 2001*a*, *b*, Tirado and Yoon 2003), coinfection has received little attention in studies of natural systems in comparison to studies examining population-level prevalence rates (but see Lello et al. 2004, Jolles et al. 2008, Seabloom et al. 2009). Our results suggest that coinfection may be more predictable across geographic gradients than pathogen prevalence; in contrast to prevalence, all host species found at a site had similar levels of coinfection. Recent theory examining drivers of coinfection and prevalence suggests that interactions among virus species within hosts (e.g., synergistic mortality) should be weak to generate these results (Seabloom et al. 2009). In addition, coinfection in the current study was well-predicted by a single environmental factor, latitude, suggesting that site-level factors, rather than host-specific or within-host interactions, are the primary drivers of coinfection in this system.

We gain insights into the regulation of coinfection across this latitudinal gradient by applying well-developed theory from community ecology to disease systems (Guernier et al. 2004, Lello et al. 2004, Dove and Cribb 2006). As noted in studies of non-disease communities (Whittaker 1960, Harrison 1999), we might expect that local diversity (α diversity) may arise as a balance between the total species pool at a site (γ diversity) and turnover of species among sites (β diversity; Whittaker 1960, Harrison et al. 1992, Dove and Cribb 2006). Applying this framework to this pathogen community, we demonstrate that the increase in coinfection with latitude results in a decline in pathogen species turnover among hosts at a site. This decline in species turnover with latitude indicates a shift from dispersal-assembled within-host pathogen communities at low latitudes to niche-assembled pathogen communities at high latitudes. Such a pattern is concordant with elevated transmission rates among hosts at high-latitude sites (Condit et al. 2002, Dove and Cribb 2006, Chase 2007). Recent theoretical work motivated by the B/CYD group of vectored viruses, suggests that increased within-host richness and transmission at our northern sites could arise from higher densities of generalist vectors leading to an overall increase in intrinsic transmission rates (Seabloom et al. 2009). Further detailed sampling or replicated experiments will be required to isolate the ultimate driver of this strong coinfection gradient.

The community ecology of disease is a relatively recent focus in ecological studies, with few theoretical frameworks or empirical tests (but see Lello et al. 2004, Collinge and Ray 2006*b*, Keesing et al. 2006, Jolles et al. 2008, Seabloom et al. 2009). Our understanding of observed patterns of generalist pathogen prevalence may become more complex as we incorporate host community context. However, the field of community ecology has demonstrated the existence of simple and general patterns governing diversity of free-living organisms, such as species–area relationships or the interdependen-

cies of different components of diversity (i.e., α, β, and γ). Our current study suggests that such simple laws may also inform our understanding of pathogen diversity in natural communities.

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APPENDIX

Tables showing provenance, lifespan, and pathogen interactions of the monitored hosts; site locations, precipitation, soil nitrate, overall viral prevalence, and viruses per host averaged across three host species of four barley and cereal yellow dwarf viruses in 26 Pacific Coast grasslands; among-host comparisons in prevalence and viral species diversity in infected hosts; environmental drivers of prevalence and viral species diversity averaged across three host species; and effects of soil nitrogen on prevalence and viral species diversity averaged across three host species (*Ecological Archives* E091-052-A1).

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Appendix A. Tables showing provenance, lifespan, and pathogen interactions of the monitored hosts; site locations, precipitation, soil nitrate, overall viral prevalence, and viruses per host averaged across three host species of four barley and cereal yellow dwarf viruses in 26 Pacific Coast grasslands; among-host comparisons in prevalence and viral species diversity in infected hosts; environmental drivers of prevalence and viral species diversity averaged across three host species; and effects of soil nitrogen on prevalence and viral species diversity averaged across three host species.

TABLE A1. Provenance, lifespan, and pathogen interactions of the monitored hosts. All hosts are from the subfamily Pooideae.

	Avena Fatua	*Bromus hordeaceus*	*Elymus glaucus*
Life span	Annual	Annual	Perennial
Provenance	Exotic	Exotic	Native
Vector fecundity (aphids day^{-1}) [a]	1.08	1.03	0.52
Vector preference (aphids g^{-1} of host) [b]	14.41	17.86	2.62
Pathogen-induced reduction in fecundity [c]	0.41	0.39	0.23
Pathogen-induced reduction in among-year survival [d]	NA	NA	0.12
Tribe within the Poaceae	Poeae	Bromeae	Hordeinae

[a] Aphid production based on experimental out-plants (Borer et al. 2009).
[b] Aphid density in foraging preference trials adjusted for host size (grams of dry tissue) (Borer et al. 2009).
[c] Reduction in fecundity in inoculated plants (C. E. Mitchell, *unpublished data*).
[d] Increase in among-year mortality in inoculated plants (Malmstrom et al. 2005, Borer et al. 2007)

TABLE A2. Site locations, precipitation, soil nitrate, overall viral prevalence, and viruses per host average across three host species of four barley and cereal yellow dwarf viruses in 26 Pacific Coast grasslands.
Prevalence and species diversity are averaged across three host grasses: *Avena fatua*, *Bromus hordeaceus*, and *Elymus glaucus*.

State/ Province	County	Reserve	Site	Overall infection proportion	Viral species per host	Latitude	Longitude	2006 precipitation (mm)	Soil nitrate (ppm)
British Columbia	Vancouver Island	NA	Cowichan Annual	0.300	2.167	48.810	-122.369	1285	NA
British Columbia	Vancouver Island	NA	Cowichan Perennial	0.050	3.000	48.810	-122.369	1285	NA
Oregon	Polk	Baskett Slough	Smithfield Rd.	0.200	1.500	44.962	-122.738	1448	10
Oregon	Polk	Baskett Slough	Baskett Butte	0.105	2.500	44.962	-122.738	1448	10

Oregon	Benton	W. L. Finley	Bruce Rd.	0.350	2.286	44.421	-122.676	1094	10
Oregon	Benton	W. L. Finley	Quarry	0.700	2.071	44.421	-122.676	1094	10
Oregon	Benton	W. L. Finley	Pigeon Butte	0.250	2.200	44.421	-122.676	1094	10
Oregon	Benton	Fort Hoskins	Collins	0.475	1.786	44.408	-122.722	1094	10
Oregon	Linn	Horserock Ridge	IAE	0.100	1.500	44.107	-122.926	1504	NA
California	Yuba	Sierra Foothill	Koch Rd6	0.000	NA	39.294	-121.288	1001	2
California	Yuba	Sierra Foothill	Koch K6	0.100	1.000	39.285	-121.289	1001	5
California	Yuba	Sierra Foothill	Camp	0.143	1.500	39.256	-121.280	1001	6
California	Mendocino	Hopland	Riley	0.050	1.500	39.015	-123.060	1029	2
California	Mendocino	Hopland	Foster	0.000	NA	39.003	-123.090	1029	1
California	Mendocino	Hopland	Lake	0.050	1.000	38.995	-123.070	1382	2
California	Yolo	McLaughlin	Reservoir	0.050	1.333	38.857	-122.362	1067	2
California	Yolo	McLaughlin	Middle	0.033	1.000	38.853	-122.366	1067	2
California	Napa	McLaughlin	Mine Pit	0.050	2.000	38.842	-122.356	1067	4
California	Monterey	Hastings	Native	0.150	1.800	36.387	-121.554	627	3
California	Monterey	Hastings	Schoolhouse	0.150	1.611	36.385	-121.548	627	2
California	Monterey	Hastings	Martin Road	0.150	2.000	36.379	-121.549	627	3
California	Santa Barbara	Sedgwick	Figueroa	0.509	1.444	34.718	-120.050	434	6
California	Santa Barbara	Sedgwick	Lisque	0.500	1.178	34.718	-120.050	434	8
California	Santa Barbara	Sedgwick	Middle	0.217	1.254	34.718	-120.050	434	8
California	Orange	Irvine Ranch	Irvine Annual	0.451	1.429	33.751	-117.683	224	10
California	Orange	Irvine Ranch	Irvine Perennial	0.250	1.286	33.751	-117.683	224	10

TABLE A3. Among host comparisons in prevalence and viral species diversity in infected hosts. The final model includes only significant terms based on backwards selection[a].

Response	Source	Estimate	Std. Error	*t* value	*P*
Prevalence (df = 49)	Intercept	-12.669	2.811	-4.506	0.000
	Avena vs. *Bromus* hosts	0.924	0.306	3.020	0.004
	Perennial vs. annual hosts	0.245	0.159	1.544	0.130
	Latitude (degrees)	0.411	0.093	4.408	0.000
	Precipitation in 2006 (mm)	-0.005	0.001	-5.021	0.000
	Avena × Peren. Grass Cover	-0.051	0.032	-1.591	0.119

	Bromus × Peren. Grass Cover	0.044	0.019	2.270	0.028
	Elymus × Peren. Grass Cover	-0.029	0.015	-1.943	0.059
α diversity (strains per host; df = 40)	Intercept	-0.977	0.433	-2.257	0.030
	Latitude (degrees)	0.037	0.011	3.396	0.002

[a]Total aboveground biomass, host richness, cover of non-hosts, and cover of perennial grasses were included in the full model, but were not significant in any final models.
Nonsignificant contrasts were left in the final model if one of the contrasts was significant.

TABLE A4. Environmental drivers of prevalence and viral species diversity (α, β, and γ) averaged across three host species. The final model includes only significant terms based on backwards selection[a].

Response	Source	Estimate	Std. Error	*t* value	*P*
Prevalence (df = 25)	Intercept	-10.375	2.532	-4.097	0.000
	Latitude (degrees)	0.337	0.085	3.989	0.001
	Precipitation in 2006 (mm)	-0.005	0.001	-4.562	0.000
α diversity (viruses per host; df = 23)	Intercept	-2.049	0.499	-4.108	0.001
	Latitude (degrees)	0.076	0.016	4.732	0.000
	Precipitation in 2006 (mm)	-0.001	0.000	-2.511	0.020
β diversity (*a*/*g*; df = 23)	Intercept	2.454	0.497	4.942	0.000
	Latitude (degrees)	-0.046	0.013	-3.661	0.001
γ diversity (viruses per site; df = 23)	Intercept	1.099	0.060	18.250	0.000

[a]Total aboveground biomass, host richness, cover of non-hosts, and cover of perennial grasses were included in the full model, but were not significant in any final models. Nonsignificant contrasts were left in the final model if one of the contrasts was significant.

TABLE A5. Effects of soil nitrogen (nitrate) on prevalence and viral species diversity (α, β, and γ) averaged across three host species.
Nitrate data were missing at three sites, and so were not included in the full regression models in Tables A3 and A4.

Response	Source	Estimate	Std. Error	*t* value	*P*

Prevalence (df = 22)	Intercept	-2.761	0.438	-6.306	0.000
	Soil nitrate (ppm)	0.233	0.059	3.932	0.001
α diversity (viruses per host; df = 20)	Intercept	0.270	0.127	2.131	0.046
	Soil nitrate (ppm)	0.031	0.017	1.830	0.083
β diversity (α/γ; df = 20)	Intercept	0.669	0.157	4.263	0.000
	Soil nitrate (ppm)	0.001	0.022	0.030	0.976
γ diversity (viruses per site; df = 20)	Intercept	0.983	0.148	6.650	0.000
	Soil nitrate (ppm)	0.02044	0.02007	1.019	0.321

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