

1 **Running head:** disease impacts on communities

2 **Title:** Disease decreases variation in host community structure in an old-field grassland

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20 **Abstract:** Disease may modulate variation in host community structure by modifying the
21 interplay of deterministic and stochastic processes. For instance, deterministic processes like
22 ecological selection can benefit species less impacted by disease. When disease consistently
23 selects for certain host species, this can reduce variation in host community composition. On the
24 other hand, when host communities are less impacted by disease and selection is weaker,
25 stochastic processes (e.g., drift, dispersal) may play a bigger role in host community structure,
26 which can increase variation in structure among communities. While effects of disease on host
27 community structure have been quantified in field experiments, few have addressed the role of
28 disease in modulating variation in structure among host communities. To address this, we
29 conducted a field experiment spanning three years, using a tractable system: foliar fungal
30 pathogens in an old-field grassland community dominated by the grass *Lolium arundinaceum*,
31 tall fescue. We reduced foliar fungal disease burden in replicate host communities (experimental
32 plots in intact vegetation) in three fungicide regimens that varied in the duration of fungicide
33 exposure and included a fungicide-free control. We measured host diversity, biomass, and
34 variation in community structure among replicate communities. Disease reduction generally
35 decreased plant richness and increased aboveground biomass relative to communities
36 experiencing ambient levels of disease. Despite changes in structure of the plant communities
37 over the experiment's three years, the effects of disease reduction on plant richness and biomass
38 were consistent across years. However, disease reduction did not reduce variation in host
39 community structure, providing little evidence for ecological selection by competition or other
40 deterministic processes. Instead, disease reduction tended to amplify variation in host community
41 structure among replicate communities (i.e., within fungicide treatment groups), suggesting that
42 disease diminished the degree to which host communities were structured by stochastic

43 processes. These results of experimental disease reduction both highlight the potential
44 importance of stochastic processes in plant communities and reveal the potential for disease to
45 regulate variation in host community structure.

46 **Key words:** community assembly, fungal pathogens, plant disease, biomass, diversity

47 **Introduction:**

48 Disease can be an agent of ecological selection (sensu Vellend 2010) and thereby
49 influence the structure of host communities (Minchella and Scott 1991, Hatcher et al. 2006,
50 Mordecai 2011, Friesen et al. 2020). Field experiments reducing disease in communities have
51 revealed that disease can not only increase local species richness, but also can shift community
52 composition (Allan et al. 2010, Heckman et al. 2016, 2017, Wilfahrt et al. 2020). Relative to
53 such effects of disease on local host community structure, disease impacts on variation in
54 structure among host communities and over time have been investigated less (but see Heckman
55 et al. 2016, 2017, Cappelli et al. 2020, Szefer et al. 2020, Wilfahrt et al. 2020). Thus, it remains
56 unresolved to what degree disease plays a deterministic role in host community assembly.
57 Quantifying these disease impacts on host communities over time is important for understanding
58 how disease interacts with other processes that may drive host community assembly.

59 Communities are structured both by deterministic and stochastic processes (Chase 2007,
60 Vellend 2010, Germain et al. 2013). To understand how disease affects host community
61 composition, it may be important to explore how disease alters the relative importance of
62 deterministic and stochastic processes. In host communities in which interspecific competition is
63 an agent of ecological selection, disease can decrease the degree to which the community is
64 dominated by better competitors and shift the relative abundance of certain host taxa (Hatcher et

65 al. 2006, Mordecai 2011). However, variation among host communities in species composition
66 can lead to variation among communities in selection by natural enemies, impeding host
67 communities from converging towards a consistent set of species (Lind et al. 2013). Ecological
68 selection, such as by disease, can also reduce the relative influence of stochastic demographic
69 processes, such as dispersal or ecological drift, on community structure (Chase et al. 2009,
70 Vellend 2010). Over time or across multiple local communities, stochastic processes can increase
71 variation in community structure (Germain et al. 2013, Gilbert and Levine 2017). Thus, to the
72 degree that disease reduces the relative influence of stochastic processes within host
73 communities, disease may reduce variation in community structure over time and among
74 communities. Consequently, disease impacts on host communities may depend on the interplay
75 of multiple processes, both deterministic and stochastic, that drive community structure. More
76 broadly, disease may increase, have no effect on, or decrease variation in host community
77 structure, depending on the relative importance of disease, along with other agents of ecological
78 selection, and stochastic processes that structure communities.

79 The role of disease in structuring host communities may also depend on the structure of
80 the parasite community. Hosts are commonly infected with a diverse community of parasite
81 species that vary in their effects on host fitness and growth (Friesen et al. 2017), so variation in
82 parasite community structure can drive disease impacts on host individuals (Mordecai et al.
83 2015) and communities (Cappelli et al. 2020, Friesen et al. 2021). Moreover, parasite abundance
84 and diversity both vary temporally. Specifically, infections are often seasonal, which will
85 generate differences in disease burdens over time and contribute to variation in parasite
86 community structure (Sapp and Esch 1994, Grunberg and Sukhdeo 2017). Previous field
87 experiments have found that parasite impacts on the host community can depend on parasite

88 community composition (Cappelli et al. 2020, Friesen et al. 2021) and that parasite community
89 composition can shift seasonally (Sapp and Esch 1994, Grunberg and Sukhdeo 2017, Halliday et
90 al. 2017). But, the effect of seasonal variation in parasite community composition on plant
91 community structure remains untested.

92 Here, we manipulated foliar fungal disease burden by treating plant communities with
93 fungicide for different periods of the growing season, and then tested the effects of foliar fungal
94 disease on changes in plant community structure and biomass over three years. At the beginning
95 of our experiment, plant communities were dominated by the perennial grass tall fescue, which is
96 host to a diverse community of foliar fungal diseases that vary in infection prevalence over the
97 growing season (Halliday et al. 2017). Our seasonal fungicide treatments were designed to alter
98 the species composition of the parasites that cause these different diseases. The fungicide
99 treatments ended up having little effect on parasite species composition (unpublished data), but
100 substantially reduced overall disease burden, so here we focus on the effects of disease burden on
101 host communities. To investigate the interplay of deterministic and stochastic processes, we
102 quantified not only effects of disease on the species richness, composition, and biomass of local
103 host communities, but also their impacts on variation among communities over time.

104 **Methods:**

105 Experimental design

106 Our experiment was conducted at Widener Farm in the Duke Forest Teaching and
107 Research Laboratory in Orange Co., North Carolina, USA. This old field site was previously
108 used to produce crops until 1992. Since then, the site has been mowed at least once a year in the
109 summer to maintain dominance of the plant community by herbaceous plants. Typically, four

110 non-native perennial species, *Lespedeza cuneata*, *Lonicera japonica*, *Sorghum halepense*, and
111 *Lolium arundinaceum* (= *Festuca arundinacea*, tall fescue) constitute the majority of community
112 cover (Heckman et al. 2016). Of these plant species, tall fescue was the most abundant in our
113 plots (Figure S1) and supports both a heavy load of foliar fungal disease and a great diversity of
114 foliar fungal parasites (Halliday et al. 2019). So, we used observed epidemics of foliar fungal
115 disease on the abundant host tall fescue to design our experimental treatments to shift parasite
116 community composition on that host.

117 Epidemics of foliar fungal disease on tall fescue are seasonal; typically, the disease
118 anthracnose (caused chiefly although not exclusively by *Colletotrichum cereale*) peaks in late
119 spring, while brown patch (caused by *Rhizoctonia solani*) peaks in the summer and crown rust
120 (caused by *Puccinia coronata*) peaks in the early fall (Figure S2, Halliday et al. 2017).
121 Hereafter, we refer to these diseases rather than the causal parasite species because our study
122 focuses on disease impacts. We designed different fungicide treatment regimens to correspond
123 with the peak of the seasonal epidemics of these diseases, so that different fungicide treatments
124 would shift the composition of disease infecting tall fescue. Treatments varied in their duration
125 of fungicide exposure including: no fungicide (control), fungicide until mid-July (approximately
126 7 months, soon after the typical start of brown patch epidemics), fungicide until mid-September
127 (approximately nine months, soon after the typical start of crown rust epidemics), and year-round
128 application of fungicide. Fungicide application started each year in January, except in 2017,
129 when fungicide treatments started in May. Throughout, we refer to these fungicide treatments
130 with respect to their designated yearly duration of fungicide application: never, seven months,
131 nine months, and year-round. Fungicide treatments started in May 2017 and ended in February
132 2020.

133 In total, we established 64 experimental plots in an intact old-field vegetation site that
134 was fenced to exclude vertebrates, particularly deer and rodents. At the beginning of the
135 experiment on 11-13 June 2017, we increased tall fescue dominance of plant communities by
136 clipping shoots of *Lespedeza cuneata* and *Sorghum halepense* at their base. Experimental plots
137 were assigned to one of four fungicide treatments in a fully randomized design. Each plot was 2
138 m × 2 m and separated by 1 m with a 2-m buffer surrounding the entire experimental area, and
139 the 64 plots were arranged in a 16×4-plot array with a total footprint of 51 m × 15 m.

140 The fungicide *Dithane75DF Rainshield*, (75% mancozeb, Dow AgroSciences,
141 Indianapolis, Indiana, USA) was used to reduce foliar fungal disease. Prior studies have found
142 that application of this fungicide at recommended rates did not affect plant growth or
143 mycorrhizal colonization (Parker and Gilbert 2007), including growth of tall fescue and other
144 common species from our experimental site (Heckman et al. 2016). Fungicide was applied
145 approximately every two weeks during the designated fungicide treatment time. Plots not
146 assigned to receive fungicide treatments were instead sprayed with water. In 2018, plots were
147 washed with water at the end of each seasonal treatment's fungicide application period (i.e., in
148 mid-July for the seven-month treatment and mid-September for the nine-month treatment) to
149 remove fungicide from leaves and allow fungal colonization of the plots for the rest of the year.
150 Plots in all treatments were washed for the same duration to equalize water addition among plots.
151 Plots were mown annually at the end of each growing season to reduce the establishment of
152 woody vegetation, which tends to outcompete herbaceous species in Eastern North American
153 old-fields, including our site (Wright and Fridley 2010, Heckman et al. 2022)

154 Data collection

155 Plant community composition was recorded on 09 November 2017, 10 October 2018, and
156 10 October 2019. The cover of all plant species, including litter and bare ground, was visually
157 estimated within a 0.75 m x 0.75 m permanent quadrat in each plot. The absolute cover of each
158 plant species was estimated independently as plants may overlap, and thus total cover often
159 exceeded 100% in plots. We then calculated relative cover for each species in a plot as the
160 absolute cover of that species divided by the sum of absolute cover of all species in a plot.

161 We evaluated the effectiveness of the fungicide treatments by surveying disease
162 prevalence in tall fescue. Disease prevalence was not assayed for other plant species; we
163 rationalized that quantifying disease on the most abundant host species, tall fescue, which
164 initially constituted ~87% of plant cover in our plots (Figure S1.B), provides a useful estimate
165 for the overall effectiveness of the fungicide on most diseases in this system. We surveyed
166 disease monthly, starting in March 2017 and ending in December 2019, by haphazardly selecting
167 20 tillers (i.e., individual grass shoots) of tall fescue within each plot and recording visible
168 disease symptoms on all leaves on a tiller. We then quantified disease prevalence within a plot as
169 the total number of infected leaves (infected = any amount of disease) divided by all leaves
170 surveyed across the 20 plants, treating leaves as host individuals because fungal infections are
171 localized within a leaf (Halliday et al. 2017).

172 We measured aboveground plant biomass each year in mid-November by harvesting the
173 entire 0.75 m x 0.75 m quadrat in 2017, and by harvesting two 0.5 m x 0.2 m strips of vegetation
174 in 2018 and 2019. Dead vegetation from the growing season (litter), which did not include prior
175 year's biomass, was included in biomass measurements. In 2018, we sorted plant biomass into
176 five categories: tall fescue, non-fescue monocots, non-woody dicots, woody dicots, and litter.
177 Aboveground biomass was then oven-dried at 65 °C for at least 72 hours and then weighed.

178 Analysis

179 *Fungicide treatment effects on disease*

180 We quantified host population-level disease burden by calculating the annual area under
181 the disease progress stairs (AUDPS) (Simko and Piepho 2012) using the monthly disease
182 prevalence survey data. This measure of disease burden (i.e., AUDPS) provides an advantage
183 over other measures like the area under the disease progress curve because it gives a better
184 estimate of the contribution of the first and last observation (Simko and Piepho 2012). To
185 calculate disease burden, we used disease survey data from May until November of each year to
186 be consistent across years; also, this timespan represents the bulk of the growing season in our
187 system. The AUDPS, our measurement of disease burden, was estimated using the ‘agricolae’ R
188 package (Mendiburu 2021). We calculated a cross-disease disease burden that represents total
189 infection pooled across the three most prevalent diseases of tall fescue (anthracnose, brown
190 patch, and crown rust) as an indicator of overall disease burden. We also include disease burden
191 data for each of the three diseases infecting tall fescue. As a measure of parasite composition, we
192 also quantified the relative contribution of each disease’s AUDPS to the cross-species AUDPS.
193 Quantifying the relative contribution of each disease allows us to account for overall differences
194 in disease due to fungicide.

195 The effects of the fungicide treatments on disease burden over time were evaluated using
196 a repeated measures analysis of variance (ANOVA). In this analysis, we used the annual cross-
197 disease burden (i.e., AUPDS). In the ANOVA we included fungicide treatment, year, and
198 treatment*year interaction as fixed effects, and experimental plot as a random effect. As a
199 measure of disease reduction, we also report the disease burden log response ratio relative to the
200 control-no fungicide plots.

201 *Plant diversity and biomass*

202 We used repeated-measures ANOVAs to test the effects of fungicide treatment over time
203 on plant biomass and plant diversity metrics. Plant diversity was quantified using three metrics
204 based on Hill's series of diversity (Hill 1973) in the 'vegan' package (Oksanen et al. 2008). The
205 value of q in Hill's series is related to differences in the weighting of the relative abundance of
206 taxa: taxonomic richness ($q = 0$, no abundance weighting), Hill-Shannon diversity ($q = 1$,
207 provides a balanced measure of both rare and common species), and Hill-Simpson diversity ($q =$
208 2 , emphasizes common species) (Jost 2006). All ANOVAs included fungicide treatment, year,
209 and fungicide treatment*year interaction as fixed effects and experimental plot as a random
210 effect to account for repeated measures.

211 Significance tests were based on type 3 tests in the 'afex' package (Henrik Singmann et
212 al. 2021). As a measure of the effect size for each variable in a model, we report η^2_{partial} , which is
213 calculated as the ratio of the variance explained by the variable to the sum of that explained
214 variance plus the residual error variance. For each variable in a model, η^2_{partial} is bound between
215 0 to 1. We then evaluated differences among fungicide treatments and between years using
216 Tukey's Honestly Significant Difference (HSD) post-hoc tests. We checked assumptions of
217 normality and homogeneity of variance using diagnostic plots of model residuals. To minimize
218 heteroscedasticity, we log-transformed plant richness and biomass.

219 *Plant community composition*

220 Even if plant communities change in richness when experiencing lower disease, this may
221 not translate to consistent shifts in the relative abundance of certain plant species that result in
222 similar community compositions. At one end, low disease pressure could generate convergence

223 towards a similar community state. This could happen when host species respond to disease
224 reduction by consistently increasing or decreasing in abundance. Alternatively, communities may
225 fail to converge in composition when stochastic colonization and loss of species occurs over
226 time, leading to variable community structure. Thus, we also assessed whether fungicide
227 treatments influenced plant community composition over time by modelling the relative cover of
228 plant species using a multivariate generalized linear model in the ‘mvabund’ R package with a
229 negative binomial distribution (Wang et al. 2012). This analysis allows us to detect both
230 community-level and species-level responses to fungicide treatments. When considering species-
231 level responses, we used univariate tests that were adjusted for multiple comparisons through
232 resampling based on the Holm step-down procedure (Wang et al. 2012). We accounted for
233 repeated sampling of communities by restricting permutations within blocks that correspond to
234 the identity of the experimental plot using the ‘bootID’ argument ($n = 999$ permutations).
235 Patterns of plant community dissimilarity were then visualized using nonmetric
236 multidimensional scaling based on Bray-Curtis distances of plant relative cover in R package
237 ‘vegan’ (Oksanen et al. 2008).

238 We expected the colonization and loss of plant species in our experimental plots to
239 contribute to variation in community structure among plots over time, and we predicted that
240 fungicide treatments would interact with this variation. Specifically, disease reduction could
241 further increase variability in community structure by reducing selection via disease and thus
242 increase within-treatment variation. Therefore, we also examined whether within-treatment
243 variation in community structure, measured as the distance from the Bray-Curtis treatment
244 centroid of each year, differed among treatments and years using a repeated measures ANOVA.

245 We used experimental plot as a random effect to account for repeated measures, and log-
246 transformed distance from the centroid to account for heteroscedasticity.

247 **Results:**

248 *Fungicide treatment effects on disease burden in tall fescue*

249 The application of fungicide decreased cumulative foliar fungal disease burden over the
250 growing season, as measured across the three common diseases of tall fescue ($F_{3,60} = 176.63$, $p <$
251 0.001 , $\eta^2_{\text{partial}} = 0.89$, Table S1, Figure S3, S4). In plots that received year-round fungicide,
252 disease was reduced by 42.4% in 2017, 35.6% in 2018, and 85.9% in 2019 relative to the control
253 (Table S1). In addition, disease burdens declined from 2018 to 2019 by on average 58% ($F_{2,120} =$
254 1610.82 , $p < 0.001$, $\eta^2_{\text{partial}} = 0.96$), and the effects of fungicide treatment on disease varied
255 among years (treatment*year: $F_{6,120} = 51.76$, $p < 0.0001$, $\eta^2_{\text{partial}} = 0.72$, Figure S3). In the first
256 year of the experiment (2017), disease burdens from plots experiencing year-round fungicide
257 were significantly lower than those from plots sprayed for nine months (year-round v. nine-
258 month contrast for 2017, $p < 0.001$). In subsequent years, disease burdens between those
259 fungicide treatment groups were not statistically distinguishable from each other (year-round v.
260 nine-month 2018 contrast, $p = 0.37$; 2019 contrast, $p = 0.98$, Figure S3). Despite that decrease in
261 statistical significance and the large variation among years, overall, the treatments that applied
262 fungicide over a greater fraction of the growing season tended to reduce disease burden more
263 over the growing season.

264 The community composition of foliar diseases remained relatively consistent over
265 treatments and years. Anthracnose accounted for most of the disease in tall fescue throughout the
266 experiment and had the highest relative contribution across all fungicide treatments and years

267 (mean relative contribution = 0.93, range: 0.53 to 1.0). Rust (mean, = 0.11, range: 0.0 to 0.42)
268 and brown patch (mean = 0.07, range: 0.0 to 0.39) infections were less frequent (Figure S5).
269 Thus, although all fungicide treatments reduced disease, seasonal fungicide application did not
270 alter parasite community composition.

271 *Plant diversity*

272 Fungicide treatments generally reduced plant diversity, but with important variation
273 among diversity metrics. Plant richness varied among years (year: $F_{2,120} = 72.18$, $p < 0.001$,
274 $\eta^2_{\text{partial}} = 0.42$), and was reduced by fungicide treatment (treatment: $F_{3,60} = 3.81$, $p = 0.014$,
275 $\eta^2_{\text{partial}} = 0.07$). Plant richness was comparable in 2017 (mean richness = 4.59) and 2019 (mean
276 richness = 4.14; Tukey HSD $p = 0.089$), but relatively higher in 2018 (mean richness = 7.16;
277 Tukey HSD $p < 0.001$, Figure 1, left column of panels). Still, the effect of fungicide treatment on
278 plant richness did not change between years (treatment * year: $F_{6,120} = 1.20$, $p = 0.32$). Control
279 plots that were never sprayed with fungicide, experiencing ambient levels of disease, on average,
280 had 1.2 more plant species than plots treated with fungicide (Figure 1, left column of panels).
281 Plant richness did not differ between the three fungicide-treated groups (Tukey HSD $p > 0.05$).
282 In summary, fungicide reduced plant richness independently of the duration of fungicide
283 exposure, and the effect of fungicide on richness was consistent across years despite changes in
284 plant richness.

285 Fungicide treatment reduced plant Hill-Shannon diversity ($F_{3,60} = 5.42$, $p = 0.002$, η^2_{partial}
286 $= 0.12$) and Hill-Simpson diversity, although this latter main effect was only marginally
287 significant ($F_{3,60} = 2.68$, $p = 0.06$, $\eta^2_{\text{partial}} = 0.07$). Between-year variation in both Hill-Shannon
288 and Hill-Simpson diversity was large and a main driver of plant diversity in this experiment

289 (year: Shannon $F_{2,120} = 154.44$, $p < 0.001$, $\eta^2_{\text{partial}} = 0.55$; Simpson $F_{2,120} = 100.85$, $p < 0.001$,
290 $\eta^2_{\text{partial}} = 0.44$, Figure 1). Moreover, there was a significant interaction between fungicide
291 treatment and time for both diversity measures (treatment*year: Shannon $F_{6,120} = 5.49$, $p \leq 0.001$,
292 $\eta^2_{\text{partial}} = 0.12$; Simpson $F_{6,120} = 4.19$, $p = 0.001$, $\eta^2_{\text{partial}} = 0.09$). Hill-Shannon and Hill-Simpson
293 diversity did not differ among treatment groups in 2017, the first year of experiment (Tukey
294 HSD $p > 0.05$). In 2018, both Hill diversity metrics were higher in the control plots than the
295 fungicide-treated plots, except for Hill-Simpson diversity in plots treated with fungicide year-
296 round (Tukey HSD: Figure 1). Finally, in 2019 only the control and year-round fungicide-treated
297 plots differed in plant diversity (Tukey HSD: Shannon $p = 0.014$, Simpson $p = 0.017$). In
298 contrast to plant richness, the effects of fungicide on Hill-Shannon and Hill-Simpson diversity
299 were more variable among fungicide treatment groups, and only became evident after the first
300 year of the experiment.

301 *Plant biomass*

302 Plant community biomass increased in response to the fungicide treatments (treatment:
303 $F_{3,60} = 20.27$, $p < 0.001$, $\eta^2_{\text{partial}} = 0.25$) (Figure 2). Year-round fungicide treatment increased plot
304 biomass by 14.9% in 2017, 47.7% in 2018, and 46.6% in 2019 relative to the control (i.e., no
305 fungicide) plots. Similar increases in plant biomass also occurred in the plots treated with
306 fungicide for nine months (% percent change relative to control: 2017 = 12.1%, 2018 = 44.4%,
307 2019 = 45.3%). Increases in plant biomass from plots exposed to fungicide for seven months
308 were about half as large as those from the other fungicide treatments (percent change relative to
309 control: 2017 = 5.1%, 2018 = 26.7%, 2019 = 26.5%). Similar fungicide treatment effects were
310 observed in the 2018 sorted biomass data, and differences in 2018 plant biomass were chiefly
311 driven by an increase in fescue biomass within fungicide-treated plots (Figure S6). Across all

312 treatments, plant biomass increased over time and was highest in 2019 (year: $F_{2,120} = 64.14$, $p <$
313 0.001 , $\eta^2_{\text{partial}} = 0.42$). There was weak evidence of an interaction between fungicide treatment
314 and time on plant biomass, which may have resulted from an increase in fungicide effects on
315 biomass in 2018 and 2019 (treatment*year: $F_{6,120} = 1.99$, $p = 0.07$). Overall, differences in plant
316 community biomass were related to variation in fungicide exposure throughout the growing
317 season, so that communities exposed to fungicide for a longer duration had greater biomass.

318 *Plant community composition*

319 Plant communities did not converge towards a similar community composition. The
320 species-level composition of plant communities changed over time (multivariate glm: deviance=
321 34.73 , $p < 0.001$), but was not affected by fungicide (treatment: deviance= 26.46 , $p = 0.897$,
322 Figure S7, S8). Additionally, the temporal changes in plant community composition did not
323 interact with the fungicide treatments (treatment *time: deviance = 4.85 , $p = 0.718$). For
324 example, the relative cover of the dominant plant, tall fescue, was not affected by fungicide
325 treatments ($p = 0.45$), but generally declined over time (univariate test, $p > 0.001$). In the first
326 year of the experiment in 2017, all plots were dominated by tall fescue (mean relative % cover =
327 87.48 , $sd = 8.59$), then fescue declined in 2018 (mean relative % cover = 62.37 , $sd = 16.31$) and
328 2019 (mean relative % cover = 69.49 , $sd = 20.30$). Similarly, tall fescue absolute percent cover
329 was not impacted by fungicide treatments and declined over time (mean absolute % cover:
330 2017= 95.9 , 2018 = 83.0 , 2019 = 82.4), so these results were not driven by differences in total
331 absolute percent cover of all species. We could not identify any plant species that differed in
332 relative abundance between fungicide treatments over time (all univariate species-level tests: p -
333 adjusted > 0.05). Ultimately, the fungicide treatments did not affect species-level composition of

334 plant communities, indicating communities were not converging towards a similar community
335 state under lower disease.

336 Although fungicide treatments did not affect species-level composition of plant
337 communities, the fungicide treatments tended to increase variation in plant community
338 composition (treatment: $F_{3,60} = 11.05$, $p < 0.001$ $\eta^2_{\text{partial}} = 0.260$). Plant community composition
339 varied more among plots within the year-round fungicide treatment and more among plots within
340 the nine-month fungicide treatment than within the seven-month and never-sprayed fungicide
341 treatment groups (Tukey HSD $p < 0.05$, Figure 3). In addition, variation in plant community
342 composition among plots increased over time (year: $F_{2,120} = 307.01$, $p < 0.001$ $\eta^2_{\text{partial}} = 0.651$),
343 and the effect of fungicide treatments on variation in plant community composition changed over
344 time (treatment*year $F_{6,120} = 3.55$, $p = 0.004$, $\eta^2_{\text{partial}} = 0.061$). This interaction was driven by
345 greater increases over time in variation among the plots sprayed with fungicide for nine months,
346 and among the plots sprayed year-round (Tukey HSD, Figure 3). This indicates that these
347 disease-reduction treatments amplified variation in host community structure over time. While
348 fungicide treatments did not explain differences in the average plant community composition,
349 variation in community composition within fungicide treatment groups was greater in plots
350 treated with fungicide for a longer time each growing season.

351 **Discussion:**

352 Foliar fungal infections are ubiquitous, and yet their effects on plant community structure
353 among communities and over time are not well known. Here, experimentally reducing foliar
354 fungal disease in intact plant communities generally decreased plant diversity and increased plant
355 biomass. However, disease reduction did not lead to consistent shifts in plant composition and

356 communities did not converge towards a similar composition under lower disease. Instead, plant
357 community composition varied considerably among experimental plots and over time, and this
358 among-plot variation in plant composition was amplified by disease reduction. Overall, our
359 three-year experiment suggests that foliar fungal diseases can not only maintain or change plant
360 diversity, but can modulate the spatiotemporal dynamics of plant community composition.

361 Variation in disease did not explain differences in average plant community composition
362 among plots (indicating no community convergence within treatment groups), but played a
363 notable role in reducing variation among communities. Consistent shifts in plant species
364 composition and abundance in response to disease reduction did not occur in our field
365 experiment. Consequently, community assembly did not appear to be deterministic. Among our
366 plots, stochastic colonization and extinction of plant species occurred, as evidenced by the high
367 variation in community composition among plots throughout the experiment. Thus, although
368 plant communities responded to the fungicide treatments, as shown by richness and Hill-
369 diversity metrics, plant species did not respond in a consistent way that would result in
370 convergence of plant communities towards a similar community state within a treatment group.
371 Disease reduction may not favor specific plant species in this system, or we could not detect
372 species-level selection given the large heterogeneity in community composition. Plant
373 community composition was variable among experimental plots and this variation could be
374 related to dispersal limitation in our system (Martin and Wilsey 2012, Collins et al. 2017) along
375 with a tendency for communities to exhibit ecological drift. In contrast to any signals of
376 community convergence in response to disease exclusion, plant community composition was
377 more variable among plots that were treated with the fungicide. Being released from disease
378 appears to amplify variation in community structure in our system. Weaker selection can

379 heighten the importance of other processes that govern community structure, so being released
380 from disease may have made communities more influenced by stochastic processes. Herbivore
381 and predator exclusion experiments have reported similar findings to our study and may point to
382 a broader role of natural enemies in reducing variation in community composition by reducing
383 the relative contribution of stochastic processes (Chase et al. 2009, Mortensen et al. 2018, Chen
384 et al. 2022).

385 Over the three years of this experiment, plant communities changed considerably, starting
386 as primarily dominated by tall fescue and becoming relatively more diverse. These shifts in plant
387 communities may have resulted in part from a shift in the timing of annual mowing: the
388 experiment was mowed the summer before the experiment was implemented (i.e., in 2016), and
389 thereafter, was mowed during the late fall. Mowing later in the year may have allowed species
390 other than tall fescue, particularly dicots, to grow undisturbed through the summer and fall. This
391 could potentially increase plant establishment from seed, survivorship, growth from root stocks,
392 and size at the end of the growing season, all of which could have led to increased plant diversity
393 over time. Despite changes in plant communities over time, disease reduction consistently
394 lowered plant richness in our experiment. In contrast, Hill-Shannon and Hill-Simpson diversity
395 metrics, which place weight on species abundance, revealed treatment effects that varied over
396 time. In the first year of the experiment, Hill-Shannon and Hill-Simpson diversity were similar
397 among fungicide treatment groups, and this was likely due to the dominance of tall fescue among
398 all plots. This result reflects the high initial dominance of tall fescue among all plots. It may also
399 reflect an underestimation of plant diversity in the first year, when the plant community survey
400 was performed a month later (November) than in subsequent years (October). By November,
401 some species may have senesced and not been detected. In subsequent years, after tall fescue

402 declined in both absolute and relative abundance across plots, Hill-Shannon and Simpson did
403 differ among fungicide treatments and diversity was generally lower in fungicide-treated plots.
404 Foliar fungal disease may increase (as shown here), decrease, or have no effect on plant diversity
405 (Peters and Shaw 1996, Mitchell 2003, Allan et al. 2010). These effects of disease on host
406 diversity may interact with host temporal dynamics and further alter the trajectory of host
407 community assembly (Kardol et al. 2006, Jiang et al. 2020, Szefer et al. 2020, Wilfahrt et al.
408 2020, Heckman et al. 2022). In our system, the initial decline of the dominant grass, tall fescue,
409 along with the colonization and extinction of other plants that were potentially released from
410 disease may have resulted in the observed effects on plant diversity over time.

411 Plants are exposed to a diverse array of parasites that impact their fitness (Mordecai
412 2011, Bever et al. 2015), so plant diversity may further be affected by changes in the parasite
413 composition (Cappelli et al. 2020). Variation in fungicide impacts could occur via additive
414 effects of disease burdens (Dantec et al. 2015) and/or shifts in parasite composition (Cappelli et
415 al. 2020). In addition, differences in parasite community structure can alter within-host
416 interactions that scale up to impact disease epidemics (Halliday et al. 2017, Clay et al. 2020). In
417 our experiment, treating plant communities with fungicide for a longer time generally reduced
418 disease more. However, differences in the overall disease burden among plots in different
419 fungicide treatments did not result in further differences in plant richness among treatment
420 groups. Despite timing the fungicide treatments to fit the phenology of the foliar fungal parasites
421 in our system, the treatments had little effect on parasite community composition, so our
422 experiment was not able to test effects of parasite community composition on host diversity.
423 While we could not test effects of parasite community composition, the reduction of plant
424 diversity under fungicide treatment, with relatively little variation in effects among the three

425 fungicide treatments, suggests that the effects of fungicide treatment on plant diversity were
426 mediated by something shared among fungicide treatments. For example, in all treatments,
427 fungicide reduced disease in spring and early summer, key seasons for growth of tall fescue.

428 Interactions between plant communities and disease are reciprocal in nature; disease
429 plays a role in mediating host diversity (Minchella and Scott 1991, Allan et al. 2010, Mordecai
430 2011) and at the same time disease transmission and diversity are also driven by host community
431 structure (Halliday et al. 2019, 2021). These feedbacks between disease and host communities
432 may occur on different timescales (Halliday et al. 2019). For example, in our system, disease
433 diversity and abundance tend to peak later in the growing season (Halliday et al. 2017, Figure
434 S2), while some disease impacts on host communities (e.g., richness effects) appear to occur
435 earlier, in spring and early summer, as indicated by the similar effects of the different fungicide
436 treatments on plant richness. Therefore, differences in disease burden/abundance may not be
437 directly related to disease impacts on host communities. Scenarios like this may occur if disease
438 diversity or abundance peaks later or earlier than critical time points of host growth or
439 colonization, which help shape communities. Moving forward, incorporating more temporal
440 components, such as varying fungicide treatments within a growing season, into studies on the
441 feedback between disease and communities (Halliday et al. 2019) may provide further insight
442 into these complex dynamics.

443 Along with its impacts on host diversity, disease can reduce plant biomass and contribute
444 to variation in ecosystem productivity (Mitchell 2003, Allan et al. 2010, Seabloom et al. 2017).
445 Here, exposure to fungicide and consequently lower levels of disease throughout the growing
446 season generally led to greater plant community biomass. In addition, a longer duration of
447 fungicide exposure further increased plant biomass. Overall, these impacts of disease on plant

448 community biomass were strong and resulted in a ~50% decline in productivity in certain years.
449 Such prominent changes in biomass and primary productivity could have further implications for
450 other consumers in our system. The strong response of plant biomass to differences in disease in
451 our field experiment provides another important example of the effects of diseases on
452 ecosystems (Mitchell 2003, Seabloom et al. 2017, Zaret et al. 2022).

453 Similar to predators and other natural enemies (Chase et al. 2009, Chen et al. 2022),
454 parasites interact with their hosts in ways that may shape the relative importance of deterministic
455 and stochastic processes throughout community assembly. While disease reduction did not result
456 in one signature of deterministic community assembly, plant communities that converged in
457 composition, disease did reduce variation in community structure. When disease was reduced,
458 communities tended to become less diverse and more variable, suggesting that the relative
459 importance of stochastic processes may increase under lower disease (i.e., under weaker
460 selection). Given that disease could be an agent of selection in most systems, resolving its role in
461 either reducing or amplifying variation among communities may yield insights into its
462 consequences for the trajectory of community assembly. Taken together, our study suggests that
463 while disease impacts on host communities may be chiefly driven by selection in conjunction
464 with other deterministic processes like competition, how disease shapes communities over time
465 also can depend on stochastic processes structuring those communities.

466 **Data Availability Statement:** The data and code that support the findings of this study are
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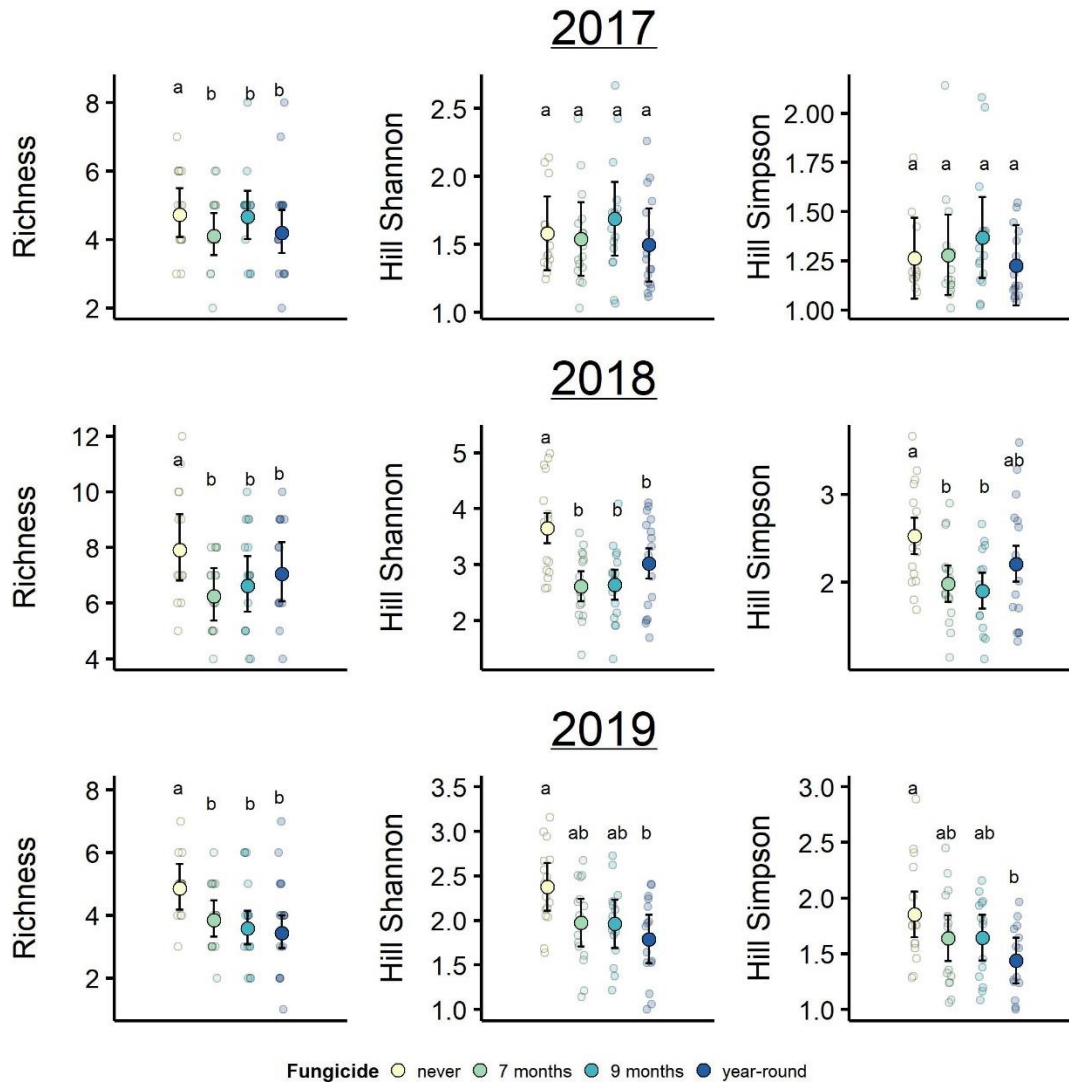
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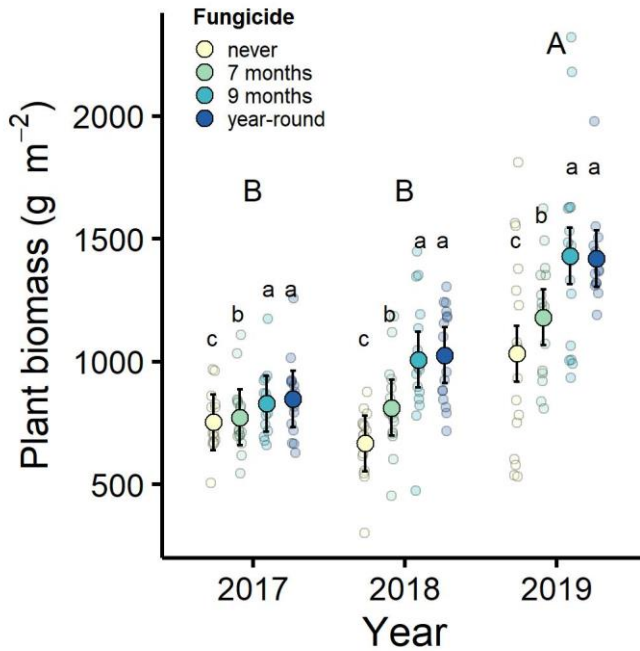
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602 **Figure 1.** Plant diversity, measured as taxonomic richness, Hill-Shannon diversity, and Hill-
603 Simpson diversity was generally reduced by fungicide treatments and varied across years. Means
604 not sharing the same lower-case letters indicate significant differences between fungicide
605 treatment groups. Due to the treatment*time interaction, Hill Shannon and Simpson diversity
606 groupings are based only on comparisons between treatments within each year. Plotted are
607 observed treatment means and their associated 95% confidence intervals, and smaller points
608 display the raw data, which were jittered for visualization.



609

610 **Figure 2.** Plant community biomass generally increased in response to fungicide treatments.
611 Years not sharing the same capital letters indicate significant differences between years based on
612 Tukey's HSD post-hoc tests. Treatments not sharing the same lower-case letters indicate
613 significant differences among fungicide treatment groups. Plotted are observed treatment means
614 and their associated 95% confidence intervals, and smaller points display the raw data, which
615 were jittered for visualization.

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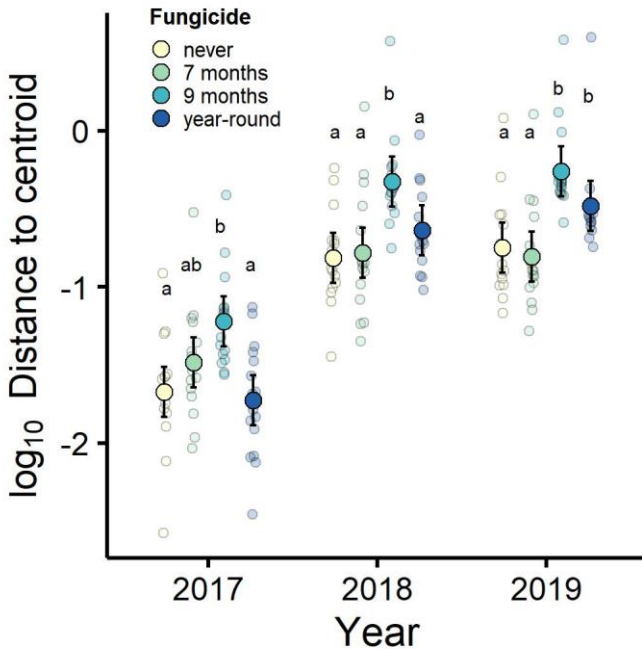
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630 **Figure 3.** Variation in plant community composition increased over time and with seasonal
631 duration of fungicide treatment. The fungicide treatments tended to increase variation in plant
632 community composition, and that increase in variation tended to amplify over time, particularly
633 in plant communities treated with fungicide for nine months out of the year or year-round.
634 Within-treatment variation in plant community composition was measured within years as the
635 log-transformed distance to the Bray-Curtis community centroid. Plotted are observed treatment
636 means on log-transformed data and their associated 95% confidence intervals, and smaller points
637 display all data, which were jittered for visualization. Treatment means not sharing the same
638 letters denote significant differences based on Tukey's post-hoc comparisons of fungicide
639 treatments within each year.

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