

## REPORT

## Endophytic fungi alter relationships between diversity and ecosystem properties

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### Abstract

Recent studies have expanded research on biodiversity by investigating whether the effects of diversity on ecosystem functioning hinge on the presence of symbiotic microorganisms. Cool-season grasses commonly harbour endophytic fungi that can enhance plant resistance to herbivory, drought and competition. We address whether these endosymbionts modify relationships between diversity and two ecosystem properties: productivity and invasibility. We develop a graphical model that predicts endophyte infection of a grass host will weaken correlations between diversity and ecosystem properties. We then use a long-term field experiment to test this prediction by manipulating symbiosis in tall fescue grass (*Festuca arundinacea*), a common and invasive species in the US. As predicted, endophyte infection reduced the strength of correlations between diversity and both primary productivity and the invasiveness of tall fescue. By altering relationships between diversity and ecosystem functioning, endophytic fungi may contribute more to the dynamics of communities than previously supposed.

### Keywords

Diversity, ecosystem functioning, endophytic fungi, *Festuca*, invasibility, *Neotyphodium*, productivity.

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### INTRODUCTION

Research investigating the relationship between species diversity and ecosystem functioning has advanced our basic understanding of community dynamics, and may ultimately improve conservation by focusing attention on the processes critical to sustaining natural ecosystems (Chapin *et al.* 1998). The idea that diversity can have strong effects on ecosystem processes was first suggested, among others, by Darwin (1859) and elaborated by Elton (1958). Theoretical models and experimental tests exploring relationships between diversity and ecosystem properties have proliferated during the past decade (Schulze & Mooney 1993; Naeem 2002). Recent models predict positive correlations between diversity and both the productivity (e.g. Tilman *et al.* 1997a) and the stability of communities (Doak *et al.* 1998). Experiments have revealed important functions for diversity in some cases, including the enhancement of primary productivity (Tilman *et al.* 1997b; Hector *et al.* 1999; Mikola *et al.* 2002), nutrient retention (Tilman *et al.* 1997a), nutrient flow (Cardinale *et al.* 2002), water availability (Caldeira *et al.* 2001) and

resistance to invasion (Levine & D'Antonio 1999). Mechanisms proposed to underlie diversity–ecosystem functioning relationships include the selection effect, whereby communities with higher diversity have a greater probability of including a species with a strong effect on the community and complementarity effects, in which the addition of greater phenotypic diversity to species assemblages enhances resource utilization and fills ecological niches (Wardle 1999; Loreau *et al.* 2001).

Integrating the results from models, experiments and observations into a general theory has been challenging. Discrepancies remain regarding both the nature of diversity–ecosystem functioning relationships and the mechanisms that underlie them. Not all studies have uncovered a clear relationship between diversity and ecosystem properties (Wardle 1999), and correlations often saturate at relatively low levels of diversity (Schwartz *et al.* 2000). Furthermore, observational and experimental studies frequently give conflicting results. For example, observations often indicate that more diverse communities are more susceptible to invasion (Stohlgren *et al.* 2003), while several experiments and theoretical treatments suggest the

reverse (Levine & D'Antonio 1999; see also Levine 2000 which reconciles these disparate results for a single system).

Recent work has begun to explore whether microbial symbionts of plants influence relationships between diversity and ecosystem functioning (van der Heijden *et al.* 1998; Klironomos *et al.* 2000; Hart *et al.* 2001). These symbionts, which often go unnoticed, may explain some of the inconsistencies among studies on diversity, particularly if experimenters unintentionally exclude naturally occurring symbionts, for example, by fumigating soil. Mycorrhizal fungi have received the most attention, as mycorrhizal associations can affect the composition of plant communities (Kiers *et al.* 2000; Hartnett & Wilson 2002) and the success of invasive species (Richardson *et al.* 2000). For example, Klironomos *et al.* (2000) showed that mycorrhizae caused primary productivity to level off more quickly with increasing diversity than when mycorrhizae were absent. Similarly, other symbionts, such as nitrogen-fixing bacteria, can alter ecosystem functioning and the composition of plant communities, particularly when these symbionts modify levels of soil nitrogen or promote a dominant plant species (Vitousek *et al.* 1987; Maron & Connors 1996). Thus, microbial symbionts may have major effects on the relationship between diversity and ecosystem functioning (van der Heijden *et al.* 1998).

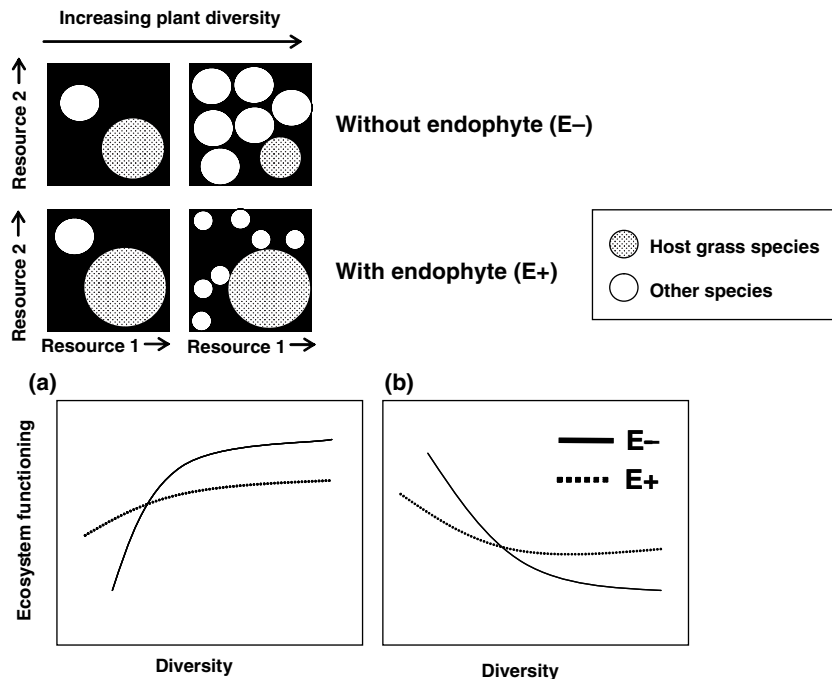
Fungal endophytes of grasses represent one class of microbial symbionts that have so far been neglected in diversity studies. These symbionts are estimated to occur in 20–30% of grass species (Leuchtman 1992) and can play important ecological roles in plant communities (Clay & Holah 1999). For cool season grasses in the subfamily Pooideae, fungal endophytes are systemic and often mutualistic (Clay 1996; Bacon & Hill 1997; but see Faeth 2002). The fungi produce mycotoxins, including several classes of alkaloids that can enhance resistance to herbivory (Clay 1996; Clay & Schardl 2002). Endophytic fungi can also increase drought resistance (Elmi & West 1995), enhance nutrient uptake (Malinowski *et al.* 2000) and improve the competitive ability of their host plant even in the absence of herbivory (Marks *et al.* 1991; Clay *et al.* 1993). In exchange for these benefits, the plant provides structure and shelter for hyphal growth, nutrients in the intercellular spaces where hyphae occur (Hinton & Bacon 1985) and a means of reproduction and dispersal when fungal hyphae grow into and infect developing seeds (i.e. vertical transmission).

Symbioses between grasses and fungal endophytes may contribute substantially to the structure and function of terrestrial communities (Clay & Holah 1999). Many prior experiments on diversity and ecosystem functioning have been conducted in temperate grasslands, using species in which fungal endophytes may be common. Therefore,

assessing the influence of these hidden symbionts on the relationship between diversity and ecosystem properties may help to clarify some of the conflicting results found in other studies.

Here, we use a field experiment with a dominant, introduced grass species to examine the question: Do endophytic fungi alter the relationship between diversity and ecosystem functioning? We examined two ecosystem properties: productivity and invasibility. In addition, we compiled data on the endophyte status of plant species commonly used in diversity experiments to facilitate the inclusion of fungal endophytes in future research.

To provide a conceptual framework for our work, we have adapted a model from Tilman *et al.* (1997b) and modified by Klironomos *et al.* (2000). In this model, plant species exploit niches along two, orthogonal resource axes (e.g. light and water), and the range of resources each species acquires is represented by a circle (Fig. 1). The addition of species to the community diminishes resources, and the effect of diversity on ecosystem functioning eventually reaches an asymptote (Tilman *et al.* 1997b). In our version of the model, when a common grass species (shown in grey) hosts a mutualistic endophyte, it can acquire more resources than when it lacks the endophyte. This enhancement of resource acquisition becomes particularly important in a diverse community because endophyte-infected grasses cause a greater reduction in the resources available to other species than do uninfected grasses. The other species then contribute less to ecosystem functioning when competing with an endophyte-infected as compared with an uninfected host. Therefore, endophyte symbiosis reduces the strength of the correlation between diversity and ecosystem functioning, regardless of whether this relationship is positive or negative. For example, consider a positive relationship between diversity and primary productivity in the absence of an endophyte (Fig. 1a). If one grass species in the community hosts a fungal endophyte, other plant species are expected to achieve lower biomass than when the grass lacks an endophyte. Therefore, other species should contribute less to productivity in the presence of an endophyte-infected grass. In communities dominated by an infected grass, a given increase in diversity would thus result in a smaller increase in productivity than in areas dominated by an uninfected grass. Similarly, because endophytes confer a competitive advantage to their host, we predict that diverse plant communities will resist invasion by an uninfected grass better than they resist invasion by an infected grass (Fig. 1b). We tested these predictions experimentally, and demonstrate that correlations between diversity and productivity, and between diversity and resistance to invasion, are altered in the presence of endophytic fungi.



**Figure 1** Graphical model depicting how mutualistic endophyte symbiosis in a common grass species can alter the relationship between species diversity and ecosystem functioning (adapted from Tilman *et al.* 1997b and Klironomos *et al.* 2000). Each species is represented by a circle that shows the amount of resources it exploits along two resource axes. Grey circles represent the grass species. If the grass hosts an endophyte, it experiences an increase in resource acquisition relative to its uninfected state. This increase will be strongest when resources are most limiting (i.e. at greater levels of species diversity). As the infected grass is superior to the uninfected grass in resource acquisition, it can more strongly reduce the amount of resources available to other species. In turn, these other species contribute less to ecosystem functioning when co-occurring with an endophyte-infected grass. Endophyte symbiosis with a grass host thus weakens the correlation between diversity and ecosystem functioning, regardless of whether this relationship is positive (a) or negative (b).

## MATERIALS AND METHODS

### Study system

A field experiment was conducted by manipulating a fungal endophyte in tall fescue (*Festuca arundinacea* Schreb.). Tall fescue is a hexaploid, perennial grass that is self-incompatible. It is native to Mediterranean Europe and Africa and was introduced to the US during the 1800s (Ball *et al.* 1993). Tall fescue is planted extensively for pasture and turf and has become invasive in some regions of the US, including Indiana (Hiebert 1990; Raloff 2003).

The fungal endophyte, *Neotyphodium coenophialum* (Latch, Christensen and Samuels) Glenn, Bacon and Hanlin, grows in the intercellular spaces of the aboveground tissues of tall fescue, where it typically accounts for <0.1% of the aboveground biomass (Hiatt & Hill 1997). The only documented means of spread is by hyphal growth into the seeds of infected plants. The endophyte produces no externally visible symptoms, but can be detected microscopically after staining thin sections of the inner leaf sheath with lactophenol cotton blue (Clark *et al.* 1983).

### Field experiment

We manipulated the presence of the fungal endophyte in tall fescue (var. KY-31) to assess the effect of symbiosis on relationships between diversity and both primary productivity (estimated by aboveground plant biomass) and the invasiveness of tall fescue (estimated by fescue biomass). As we did not explicitly manipulate diversity, we cannot infer that diversity has a direct, causal effect on ecosystem properties. However, the experiment allowed us to assess whether the symbiont altered observed correlations between diversity and ecosystem properties. The fungal endophyte was originally eliminated by long-term seed storage to create uninfected (E-) seeds from endophyte-infected (E+) seeds. Seeds used to plant the field plots were several generations removed from the original treatment and were obtained from field plots of E+ and E- plants that freely cross-pollinated (details in Clay & Holah 1999).

During the summer of 1994, alternating plots of E+ and E- tall fescue (20 m × 20 m) were established in a ploughed and tilled, old field habitat at the Indiana

University Botany Experimental Field, Bloomington, Indiana ( $n = 4$  plots per treatment; details in Clay & Holah 1999). By the fall of 1994, tall fescue seeds had germinated along with a number of grasses and forbs (both native and introduced) that recruited from the seed bank or from vegetative fragments. As plots were assigned to treatments at random, the diversity of plants was randomized with respect to the symbiosis treatment. Plots retained differences in the level of symbiosis for the duration of the experiment (Clay & Holah 1999).

Twenty subplots ( $0.5 \text{ m} \times 0.5 \text{ m}$ ), randomly located within each plot, were harvested at ground level at 6-month intervals (October 1995 to June 1998). Different subplots were harvested on each date. Harvests were sorted by species, dried and weighed to the nearest 0.01 g. We determined the Shannon–Weiner diversity index for each subplot harvested by using the biomass of each species to represent its abundance. Natural abundances of herbivores and seed predators (birds, insects, mice, rabbits, voles) were present throughout the experiment.

We analysed harvest data from the final harvest dates (Fall: October 1997 and Spring: June 1998) to focus on the endpoint of diversity–ecosystem functioning relationships. To examine the relationship between biomass estimates (total, fescue and non-fescue) and diversity, we first obtained the residuals from ANOVA models using total biomass, fescue biomass, non-fescue biomass and diversity as response variables. The models included the fixed effects of symbiosis (E+ or E–) and season (October or June), the symbiosis  $\times$  season interaction, the random effect of plot nested within the symbiosis treatment [plot(symbiosis)], and the plot(symbiosis)  $\times$  season interaction (SAS Institute Inc. 2000). This technique allowed us to remove the mean effects of these factors on both diversity and biomass estimates and to focus exclusively on the remaining correlation between diversity and ecosystem properties. Specifically, Clay & Holah (1999) showed that endophyte symbiosis in tall fescue reduced the diversity and biomass of the surrounding plant community. Therefore, we first removed these effects using the ANOVA models described above. We then examined the relationships between residual diversity and residual biomass independently of these mean effects by conducting an ANCOVA that tested whether E+ and E– treatments had significantly different correlations between residual diversity and residual biomass. In all analyses, biomass estimates were square-root transformed, and the diversity index was log-transformed to achieve normality and homoscedasticity of residuals.

### Endophytes in diversity studies

We compiled a list of grass species that have been commonly used in diversity studies by searching for papers on diversity

and ecosystem functioning published in ecological journals during the past 10 years (see references in Table 2). From those papers that included species lists as part of the experimental protocol, we recorded all species in the subfamily Pooideae. For each grass species we then searched for reports of endophyte symbioses in Farr *et al.* (1989) as well as in the Biological Abstracts (1969–2003) and Web of Science (1977–2003) electronic databases. Our searches included the species (or genus) of the plant and each of the following terms: endophyte\*, *Epichloë*, *Acremonium* or *Neotyphodium*. In addition, when data were available, we recorded the frequency of endophyte symbiosis reported in the literature, considering both the proportion of populations shown to be symbiotic and the frequency of symbiosis within populations.

## RESULTS

### Field experiment

Symbiosis between tall fescue and its fungal endophyte altered the relationship between diversity and primary productivity, as indicated by a significant symbiosis  $\times$  residual diversity interaction for total aboveground biomass of the plant community (Table 1,  $P = 0.0361$ ). The correlation between diversity and community biomass was significantly negative in the absence of the endophyte (Fig. 2a;  $r = -0.32$ ,  $P < 0.0001$ ), but non-significant when tall fescue had the endophyte ( $r = -0.12$ ,  $P = 0.13$ ). The quadratic term for diversity was non-significant ( $F_{1,314} = 0.35$ ,  $P = 0.56$ ), showing that the relationship was linear and not a saturating curve.

The endophyte also altered the correlation between diversity and the invasiveness of tall fescue, as estimated by aboveground biomass (Table 1; symbiosis  $\times$  residual diversity,  $P = 0.0018$ ). The aboveground biomass of tall fescue was more strongly negatively correlated with diversity in the absence of the endophyte than in the presence of the endophyte (Fig. 2b; E–,  $r = -0.49$ ,  $P < 0.0001$ ; E+,  $r = -0.33$ ,  $P < 0.0001$ ). As with total biomass, the quadratic term for diversity was non-significant ( $F_{1,314} = 1.36$ ,  $P = 0.24$ ).

The relationship between the biomass of non-fescue species and diversity was not significantly affected by the endophyte (Table 1; symbiosis  $\times$  residual diversity,  $P = 0.1928$ ). However, plots lacking endophytes exhibited a less positive trend between diversity and non-fescue biomass than plots with endophytes (Fig. 2c; E–,  $r = 0.59$ ,  $P < 0.0001$ ; E+,  $r = 0.71$ ,  $P < 0.0001$ ). Again, these relationships were largely linear; the quadratic term for diversity was not significant ( $F_{1,314} = 3.28$ ,  $P = 0.07$ ).

### Endophytes in diversity studies

Of the 35 Pooideae species recorded from diversity studies, 51% were known to harbour endophytic fungi

**Table 1** Field experiment: results from ANCOVA models that tested whether endophyte symbiosis in tall fescue altered the correlation between diversity and aboveground plant biomass, including all plants combined (aboveground biomass), and plants separated into tall fescue or non-fescue species. Residuals for both diversity and biomass estimates were first determined using mixed model ANOVA including the fixed effects of symbiosis (E+ or E-), season (October or June), symbiosis  $\times$  season, the random effect of plot(symbiosis), and the plot(symbiosis)  $\times$  season interaction. The mean effect of symbiosis on both diversity and biomass was removed using this approach ( $P = 1.00$ ). A significant diversity  $\times$  symbiosis interaction indicates that the correlation between residual diversity and residual biomass depends on the presence of the endophyte, independently of any mean effect of the endophyte on biomass or diversity

Effect	d.f.	Residual aboveground biomass			Residual tall fescue biomass			Residual non-fescue biomass		
		MS	F	P-value	MS	F	P-value	MS	F	P-value
Symbiosis	1	0.00	0.00	1.0000	0.00	0.00	1.00	0.00	0.00	1.0000
Residual diversity	1	78.43	16.67	<0.0001	368.82	70.54	<0.0001	672.23	217.93	<0.0001
Symbiosis $\times$ residual diversity	1	20.85	4.43	0.0361	51.76	9.90	0.0018	5.25	1.70	0.1928
Error	316	4.71	–	–	5.23	–	–	3.08	–	–

(Table 2). Only 9% of the species have been shown to be free of fungal endophytes in studies conducted so far. The endophyte status is unknown for the remaining 40% of species. An even greater number of Pooideae genera (18 of 21) used in diversity studies has been reported as infected, suggesting that many species of undetermined status may in fact host fungal endophytes. Data on the frequency of infection were available for 16 of the 18 species known to be infected. The ways in which these data were collected and reported were heterogeneous; therefore, we classified prevalence both within and among populations into three broad categories: rare (<10%), common (10–50%) and frequent (>50%). The majority of species were categorized as being commonly infected with endophytes (10–50%), both within and among populations. However, where there were multiple reports for a single species, infection frequency often varied geographically. For example, *Dactylis glomerata* was reported to be rarely infected in Finland, but frequently infected in the UK. Additional details on site-specific frequencies can be obtained from the references provided in Table 2.

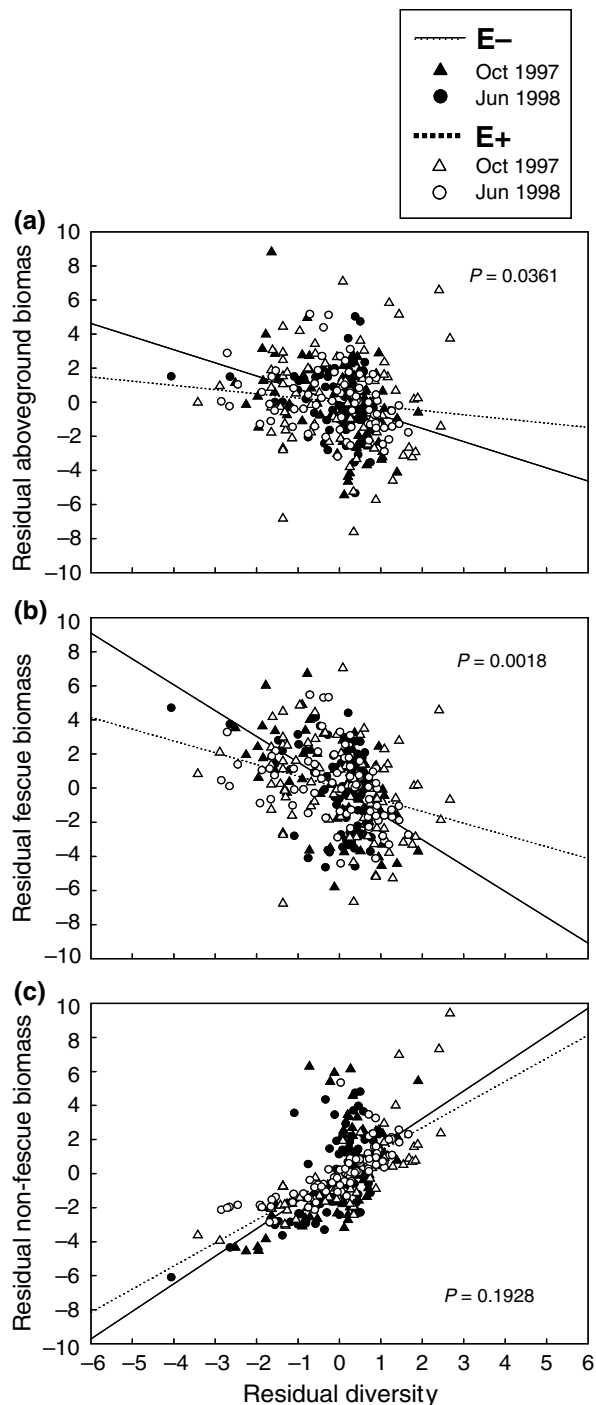
## DISCUSSION

### Does endophyte symbiosis modify the relationship between productivity and diversity?

Fungal endophytes have the potential to alter the relationship between diversity and productivity. Using a graphical model, we predicted that diversity would be less strongly correlated with ecosystem functioning when a grass was symbiotic than when the grass lacked a mutualistic endophyte (Fig. 1). This result is expected in part because endophytes both directly and indirectly enhance the competitive ability of their host grasses. In the current

study, we focused on the role of endophyte symbiosis in an introduced community dominant, tall fescue. Our field experiment to test this prediction approximated conditions following the abandonment of managed grasslands, which are common features of human-modified ecosystems.

In experimental fields, we found that the observed relationship between primary productivity and diversity was contingent upon whether tall fescue hosted the symbiotic fungal endophyte, *Neotyphodium coenophialum*. Total aboveground biomass (our estimate of primary productivity) was significantly negatively correlated with species diversity for plots containing uninfected fescue, but this relationship was non-significant for plots of symbiotic fescue (Fig. 2a). As our field experiment was not designed to detect causality in the correlation between diversity and productivity (neither factor was experimentally manipulated), this result has two possible interpretations: in the absence of the endophyte, increases in diversity may reduce productivity, or in the absence of the endophyte, increases in productivity may reduce plant diversity. Regardless of the source of causation in the absence of the endophyte, however, it is clear that the endophyte significantly altered the relationship between diversity and productivity. The mechanisms underlying this effect are currently unresolved. Most likely, the endophyte modifies diversity–productivity relationships by increasing the level of competition between non-fescue species and tall fescue. Such a change in competition is suggested by the reduced biomass of non-fescue species in plots of infected fescue as compared with plots of uninfected fescue (Clay & Holah 1999). Given the strong effect observed with tall fescue, we expect that mutualistic fungal endophytes in grasses may commonly alter both the sign and magnitude of correlations between diversity and ecosystem properties, particularly in C<sub>3</sub>-dominated grasslands.



**Figure 2** Field experiment: regression lines for residual aboveground biomass vs. residual species diversity. (a) residual total aboveground (plant community) biomass, (b) residual tall fescue biomass and (c) residual non-fescue biomass. Each symbol represents a single subplot, and data from two seasons are presented (October 1997 and June 1998;  $n = 80$  subplots/endophyte treatment/date).  $P$ -values are given for the symbiosis  $\times$  residual diversity interaction (see Table 1).

### Does endophyte symbiosis in an invader alter the relationship between diversity and invasibility?

Symbiotic mutualisms may be crucial to the success of invasive species (Richardson *et al.* 2000). Specifically, vertically transmitted fungal endophytes may confer predictable advantages to invading grasses when they accompany their host to new environments. As we predicted from our model, the relationship between diversity and the invasibility of the community was weaker for infected fescue than for uninfected fescue. Our field experiment showed that diversity was more strongly negatively correlated with the biomass of uninfected fescue than with the biomass of infected fescue (Fig. 2b). We hypothesize that this effect resulted from the competitive advantage conferred by the endophyte to its host. We cannot assign causality to diversity in this experiment because diversity was not explicitly manipulated. Therefore, although diversity may reduce fescue biomass through the selection effect, complementarity effects, or other mechanisms, it is equally possible that fescue biomass controls diversity. However, if biomass rather than diversity is causal, we arrive at the surprising conclusion that increases in the biomass of uninfected fescue are more effective at suppressing species diversity than are increases in the biomass of infected fescue. Further experiments are required to untangle the relative importance of different mechanisms underlying the correlation between species diversity and the invasiveness of tall fescue.

### Symbiosis and studies on diversity and ecosystem functioning

More than 50% of Pooideae species and 80% of genera that have been used in prior diversity studies are reported to support fungal endophytes. As much of our understanding of the importance of diversity for ecosystem functioning comes from studies in grasslands (see references in Table 2), accounting for the potentially hidden effects of endophytes may help to explain inconsistencies among previous studies and make future experiments more realistic. For example, seeds of infected grasses may lose fungal viability following long-term storage, and experiments using these seeds would fail to reproduce natural hierarchies of competition. Alternatively, seeds obtained commercially may originate from plants selected to be highly infected, while the same grass species may have more variable levels of infection in nature. Given the potential for endophytes to alter the effects of diversity on ecosystem properties, it is important to consider these symbionts when planning both experimental and observational studies.

Our experiment focused on a single endophyte host, tall fescue. Tall fescue is planted extensively in the eastern US

**Table 2** Endophyte infection status (INF) of grass species and genera in the subfamily Pooideae that are commonly used in diversity experiments. Y, the group known to be infected by systemic, endophytic fungi [*Epicblöe* spp. or *Neotyphodium* (formerly *Acremonium*) spp.]; UNK, the infection status currently unknown; NF, no infection found when the species was examined. References for infection status are in superscripts. For prevalence of infection both within and among populations, 'rare' indicates <10% infected, 'common' indicates between 10 and 50% infected and 'frequent' indicates >50% infected. Dashed lines indicate species for which infection is reported, but no prevalence data were available

Species <sup>a</sup>	Species E+?	Genus E+?	INF prevalence <sup>b</sup> among populations	Prevalence <sup>b</sup> of INF individuals	References for diversity studies
<i>Agropyron millefolium</i>	UNK	Y <sup>F</sup>			11
<i>Agrostis alba</i>	Y <sup>F,P</sup>	Y	Rare <sup>P</sup>	Rare <sup>P</sup>	13
<i>Agrostis capillaries</i>	Y <sup>F,L</sup>	Y	Common <sup>L</sup>	Common <sup>L</sup>	5
<i>Agrostis gigantea</i>	UNK	Y <sup>F</sup>			7
<i>Agrostis stolonifera</i>	Y <sup>F,G,O</sup>	Y	Rare <sup>G</sup> , frequent <sup>A</sup>	Common <sup>A</sup>	9
<i>Alopecurus pratensis</i>	NF <sup>L</sup>	Y <sup>F</sup>			2, 5
<i>Anthoxanthum odoratum</i>	Y <sup>C,G</sup>	Y	Rare <sup>G</sup>		2, 5
<i>Arrhenatherum elatius</i>	Y <sup>C</sup>	Y	–	–	2, 5
<i>Avena sativa</i>	UNK	Y <sup>G</sup>			1, 4, 5
<i>Briza media</i>	UNK	UNK			15
<i>Bromus inermis</i>	UNK	Y <sup>B,F</sup>			3, 7
<i>Dactylis glomerata</i>	Y <sup>F,G,L</sup>	Y	Common <sup>K,H</sup> , rare <sup>G,L</sup>	Common <sup>G</sup>	1, 3, 5, 7, 12
<i>Elymus canadensis</i>	Y <sup>C,D,F</sup>	Y	Frequent <sup>N</sup>	Frequent <sup>D</sup>	3, 8, 11, 13, 16, 17
<i>Elymus repens</i> ( <i>Agropyron repens</i> )	Y <sup>L</sup>	Y <sup>F</sup>	Common <sup>L</sup>	Rare <sup>L</sup>	19
<i>Festuca arundinacea</i>	Y <sup>C,D,F</sup>	Y	Frequent <sup>L,M</sup>	Frequent <sup>L</sup>	3
<i>Festuca ovina</i>	Y <sup>F,L,O</sup>	Y	Common <sup>L</sup>	Common <sup>L</sup>	3, 4, 5, 12
<i>Festuca pratensis</i> ( <i>Lolium pratense</i> )	Y <sup>F,L,O</sup>	Y	Common <sup>L</sup>	Common <sup>L</sup>	5, 7
<i>Festuca rubra</i>	Y <sup>F,G,L</sup>	Y	Common <sup>L</sup>	Common <sup>L</sup>	2, 15
<i>Holcus lanatus</i>	Y <sup>C,O</sup>	Y	Frequent <sup>E</sup>	Common <sup>E</sup>	5, 15
<i>Hordeum geniculatum</i>	UNK	UNK			5
<i>Koeleria cristata</i>	UNK	Y <sup>F</sup>			8, 11, 13, 18
<i>Koeleria pyramidata</i>	UNK	Y <sup>F</sup>			16, 17
<i>Lolium multiflorum</i>	Y <sup>I</sup>	Y	Common <sup>I</sup>		1, 5, 10
<i>Lolium perenne</i>	Y <sup>I</sup>	Y	Common <sup>I</sup>		5
<i>Pascopyrum smithii</i> ( <i>Agropyron smithii</i> , <i>Elytrigia smithii</i> )	UNK	Y <sup>C,F</sup>			8, 17
<i>Phalaris arundinacea</i>	NF <sup>L</sup>	UNK			12
<i>Phalaris brachystachys</i>	UNK	UNK			1, 5
<i>Phalaris coerulescens</i>	UNK	UNK			5
<i>Phalaris pratense</i>	UNK	UNK			12
<i>Pbleum pratense</i>	Y <sup>F,G,L</sup>	Y	Common <sup>L</sup> , rare <sup>G</sup>	Common <sup>L</sup>	3, 5, 7, 12, 20
<i>Poa annua</i>	UNK	Y <sup>F</sup>			14
<i>Poa compressa</i>	Y <sup>F</sup>	Y	–	–	7
<i>Poa pratensis</i>	Y <sup>F,G</sup>	Y	Rare <sup>G</sup>		3, 5, 8, 11, 13, 17, 19, 20
<i>Sitanion jubatum</i>	NF <sup>D</sup>	Y			6
<i>Trisetum flavescens</i>	UNK	UNK			5

<sup>a</sup>Classification from Watson, L. & Dallwitz M. J. (1992). Grass Genera of the World: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references. <http://biodiversity.uno.edu/delta/> Version: 18 August 1999. Additional synonyms from USDA, NRCS (2002), The PLANTS Database, version 3.5. <http://plants.usda.gov/>. National Plant Data Center, Baton Rouge, LA 70874-4490, USA.

<sup>b</sup>Prevalence determined from multiple methods.

for pasture and turf, covering more than  $15 \times 10^6$  ha (Ball *et al.* 1993). As this species dominates many human-altered landscapes, studies on tall fescue fill a gap in diversity–invasibility studies by investigating the effects of a high

impact invader (see Levine & D'Antonio 1999). Other grass species have also been reported to gain competitive advantages from symbiosis with endophytic fungi or to mediate ecological interactions at the community level (Clay

*et al.* 1993; Watson *et al.* 1993; Omacini *et al.* 2001). Thus, fungal endophytes may have important ecological functions in many other grassland systems. Although endophytes may not be as strongly beneficial in some grass species (Saikkonen *et al.* 1998; Faeth 2002), few experimental studies have been conducted on non-agronomic species. Many questions remain. Do endophytes enhance the persistence of rare, native grass species, thereby contributing to diversity? How much does the effect of an endophyte on the relationship between diversity and ecosystem functioning depend on the magnitude of the benefits conferred by the endophyte? And, how variable are these benefits in nature?

We are just beginning to recognize the contributions of symbionts, such as mycorrhizal fungi, nitrogen-fixing bacteria and fungal endophytes, to diversity and ecosystem functioning. Experiments that manipulate symbionts can increase the realism of studies on diversity, shifting our focus from investigating simple to more complex systems. In terrestrial communities, many grass species harbour fungal endophytes, and our results show these widespread symbionts may have a strong influence on the functioning of communities.

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#### REFERENCES

- Bacon, C.W. & Hill, N.S. (1997). *Neotyphodium/Grass Interactions*. Plenum Press, New York, NY.
- Ball, D.M., Pedersen, J.F. & Lacefield, G.D. (1993). The tall fescue endophyte. *Am. Sci.*, 81, 370–379.
- Caldeira, M.C., Ryel, R.J., Lawton, J.H. & Pereira, J.S. (2001). Mechanisms of positive biodiversity-production relationships: insights provided by delta C-13 analysis in experimental Mediterranean grassland plots. *Ecol. Lett.*, 4, 439–443.
- Cardinale, B.J., Palmer, M.A. & Collins, S.L. (2002). Species diversity enhances ecosystem functioning through interspecific facilitation. *Nature*, 415, 426–429.
- Chapin, F.S., Sala, O.E., Burke, I.C., Grime, J.P., Hooper, D.U., Lauenroth, W.K. *et al.* (1998). Ecosystem consequences of changing biodiversity – experimental evidence and a research agenda for the future. *Bioscience*, 48, 45–52.
- Clark, E.M., White, J.F. & Patterson, R.M. (1983). Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of in vitro culture of the fungus. *J. Microb. Methods*, 1, 149–155.
- Clay, K. (1996). Interactions among fungal endophytes, grasses and herbivores. *Res. Popul. Ecol.*, 38, 191–201.
- Clay, K. & Holah, J. (1999). Fungal endophyte symbiosis and plant diversity in successional fields. *Science*, 285, 1742–1744.
- Clay, K., Marks, S. & Cheplick, G.P. (1993). Effects of insect herbivory and fungal endophyte infection on competitive interactions among grasses. *Ecology*, 74, 1767–1777.
- Clay, K. & Schardl, C. (2002). Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *Am. Nat.*, 160, S99–S127.
- Darwin, C.R. (1859). *On the Origin of Species*. John Murray, London.
- Doak, D.F., Bigger, D., Harding, E.K., Marvier, M.A., O'Malley, R.E. & Thomson, D. (1998). The statistical inevitability of stability–diversity relationships in community ecology. *Am. Nat.*, 151, 264–276.
- Elmi, A.A. & West, C.P. (1995). Endophyte infection effects on stomatal conductance, osmotic adjustment and drought recovery of tall fescue. *New Phytol.*, 131, 61–67.
- Elton, C.S. (1958). *The Ecology of Invasions by Animals and Plants*. Methuen, London.
- Faeth, S. (2002). Are endophytic fungi defensive plant mutualists? *Oikos*, 98, 25–36.
- Hart, M.M., Reader, R.J. & Klironomos, J.N. (2001). Biodiversity and ecosystem function: alternate hypotheses or a single theory? *Bull. Ecol. Soc. Am.*, 82, 88–90.
- Hartnett, D.C. & Wilson, G.W.T. (2002). The role of mycorrhizas in plant community structure and dynamics: lessons from grasslands. *Plant Soil*, 244, 319–331.
- Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M.C., Diemer, M., Dimitrakopoulos, P.G. *et al.* (1999). Plant diversity and productivity experiments in European grasslands. *Science*, 286, 1123–1127.
- Hiatt, E.E.I. & Hill, N.S. (1997). *Neotyphodium coenophialum* mycelial protein and herbage mass effects on ergot alkaloid concentration in tall fescue. *J. Chem. Ecol.*, 23, 2721–2736.
- Hiebert, R.D. (1990). An ecological restoration model: application to razed residential sites. *Nat. Area J.*, 10, 181–186.
- Hinton, D.M. & Bacon, C.W. (1985). The distribution and ultrastructure of the endophyte of toxic tall fescue [*Festuca arundinacea*]. *Can. J. Botany*, 63, 36–42.
- Kiers, E.T., Lovelock, C.E., Krueger, E.L. & Herre, E.A. (2000). Differential effects of tropical arbuscular mycorrhizal fungal inocula on root colonization and tree seedling growth: implications for tropical forest diversity. *Ecol. Lett.*, 3, 106–113.
- Klironomos, J.N., McCune, J., Hart, M. & Neville, J. (2000). The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecol. Lett.*, 3, 137–141.
- Leuchtman, A. (1992). Systematics, distribution, and host specificity of grass endophytes. *Nat. Toxins*, 1, 150–162.
- Levine, J.M. (2000). Species diversity and biological invasions: relating local process to community pattern. *Science*, 288, 852–854.
- Levine, J.M. & D'Antonio, C.M. (1999). Elton revisited: a review of evidence linking diversity and invasibility. *Oikos*, 87, 15–26.
- Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A. *et al.* (2001). Ecology–biodiversity and ecosystem functioning: current knowledge and future challenges. *Science*, 294, 804–808.
- Malinowski, D.P., Alloush, G.A. & Belesky, D.P. (2000). Leaf endophyte *Neotyphodium coenophialum* modifies mineral uptake in tall fescue. *Plant Soil*, 227, 115–126.

- Marks, S., Clay, K. & Cheplick, G.P. (1991). Effects of fungal endophytes on interspecific and intraspecific competition in the grasses *Festuca arundinacea* and *Lolium perenne*. *J. Appl. Ecol.*, 28, 194–204.
- Maron, J.L. & Connors, P.G. (1996). A native nitrogen-fixing shrub facilitates weed invasion. *Oecologia*, 105, 302–312.
- Mikola, J., Salonen, V. & Setälä, H. (2002). Studying the effects of plant species richness on ecosystem functioning: does the choice of experimental design matter? *Oecologia*, 133, 594–598.
- Naeem, S. (2002). Ecosystem consequences of biodiversity loss: the evolution of a paradigm. *Ecology*, 83, 1537–1552.
- Omacini, M., Chaneton, E.J., Ghersa, C.M. & Muller, C.B. (2001). Symbiotic fungal endophytes control insect host–parasite interaction webs. *Nature*, 409, 78–81.
- Raloff, J. (2003). Cultivating weeds: is your yard a menace to parks and wild lands? *Sci. News*, 163, 232.
- Richardson, D.M., Allsop, N., D'Antonio, C.M., Milton, S.J. & Rejmanek, M. (2000). Plant invasions: the role of mutualisms. *Biol. Rev.*, 75, 65–93.
- Saikkonen, K., Faeth, S.H., Helander, M. & Sullivan, T.J. (1998). Fungal endophytes: a continuum of interactions with host plants. *Annu. Rev. Ecol. Syst.*, 29, 319–343.
- SAS Institute Inc. (2000). *SAS Version 8.1*. SAS Institute, Cary, NC.
- Schulze, E.D. & Mooney, H.A. (1993). *Biodiversity and Ecosystem Function*. Springer-Verlag, Berlin, Germany.
- Schwartz, M.W., Brigham, C.A., Hoeksema, J.D., Lyons, K.G., Mills, M.H. & van Mantgem, P.J. (2000). Linking biodiversity to ecosystem function: implications for conservation ecology. *Oecologia*, 122, 297–305.
- Stohlgren, T.J., Barnett, D.T. & Kartesz, J.T. (2003). The rich get richer: patterns of plant invasions in the United States. *Front. Ecol. Environ.*, 1, 11–14.
- Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M. & Siemann, E. (1997a). The influence of functional diversity and composition on ecosystem processes. *Science*, 277, 1300–1302.
- Tilman, D., Lehman, C.L. & Thomson, K.T. (1997b). Plant diversity and ecosystem productivity: theoretical considerations. *Proc. Natl Acad. Sci. USA*, 94, 1857–1861.
- van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T. *et al.* (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396, 69–72.
- Vitousek, P.M., Walker, L.R., Whiteaker, L.D., Muellerdombois, D. & Matson, P.A. (1987). Biological invasion by *Myrica faya* alters ecosystem development in Hawaii. *Science*, 238, 802–804.
- Wardle D.A. (1999). Is “sampling effect” a problem for experiments investigating biodiversity–ecosystem function relationships? *Oikos*, 87, 403–407.
- Watson, R.N., Prestidge, R.A. & Ball, O.J.P. (1993). Suppression of white clover by ryegrass infected with *Acremonium* endophyte. In: *Proceedings of the Second International Symposium on Acremonium / Grass Interactions* (eds Hume, D.E., Latch, G.C.M. & Easton, H.S.). AgResearch Grasslands Research Centre, Palmerston North, Queensland, pp. 218–221.
- <sup>B</sup>Brem, D. & Leuchtman, A. (2001). Transition of a pathogenic endophyte to a mutualist by host shift. *Phytopathology* 91, S104 (abstract).
- <sup>C</sup>Clay, K. (1989). Clavicipitaceous endophytes of grasses: their potential as biological control agents. *Mycol. Res.*, 92, 1–12.
- <sup>D</sup>Clay, K. (1997). Fungal endophytes, herbivores and the structure of grassland communities. In: *Multitrophic Interactions in Terrestrial Systems* (eds Gange, A.C. & Brown, V.K.). Blackwell, Oxford, pp. 151–169.
- <sup>E</sup>Clay, K. & Brown, V.K. (1997). Infection of *Holcus lanatus* and *H. mollis* by *Epicblöe* in experimental grasslands. *Oikos*, 79, 363–370.
- <sup>F</sup>Farr, D.F., Bills, G.F., Chamuris, G.P. & Rossman, A.Y. (1989). *Fungi on Plants and Plant Products in the United States*. The American Phytopathological Society, St Paul, MN.
- <sup>G</sup>Koponen, H. & Makela, K. (1976). *Phyllachora graminis*, *Phyllachora silvatica*, *Epicblöe typhina*, and *Acrosporum graminum* on grasses in Finland. *Karstenia*, 15, 46–55.
- <sup>H</sup>Large, E.C. (1954). Surveys for choke (*Epicblöe typhina*) in cocksfoot seed crops, 1951–53. *Plant Pathol.*, 3, 6–11.
- <sup>I</sup>Latch, G.C.M., Christensen, M.J. & Hickson, R.E. (1988). Endophytes in annual and hybrid ryegrasses. *NZ J. Agr. Res.*, 31, 57–64.
- <sup>J</sup>Lewis, G.C., Ravel, C., Naffaa, W., Astier, C. & Charmet, G. (1997). Occurrence of *Acremonium* endophytes in wild populations of *Lolium* spp. in European countries and a relationship between level of infection and climate in France. *Ann. Appl. Biol.*, 130, 227–238.
- <sup>K</sup>Pfender, W.F. & Alderman S.C. (1999). Geographical distribution and incidence of orchardgrass choke, caused by *Epicblöe typhina*, in Oregon. *Plant Dis.*, 83, 754–758.
- <sup>L</sup>Saikkonen, K., Ahlholm, J., Helander, M., Lehtimäki, S. & Niemeläinen, O. (2000). Endophytic fungi in wild and cultivated grasses in Finland. *Ecography*, 23, 360–366.
- <sup>M</sup>Shelby, R.A. & Dalrymple, L.W. (1987). Incidence and distribution of the tall fescue endophyte in the United States. *Plant Dis.*, 71, 783–785.
- <sup>N</sup>Vinton, M.A., Kathol, E.S., Vogel, K.P. & Hopkins, A.A. (2001). Endophytic fungi in Canada wild rye in natural grasslands. *J. Range Manage.*, 54, 390–395.
- <sup>O</sup>White, J.F. Jr & Baldwin, N.A. (1992). A preliminary enumeration of grass endophytes in west central England. *Sydowia*, 44, 78–84.
- <sup>P</sup>White, J.F. Jr, Halisky, P.H., Sun, S., Morgan Jones, G. & Funk C.R. Jr (1992). Endophyte host associations in grasses. XVI Patterns of endophyte distribution in species of the tribe Agrostideae. *Am. J. Bot.*, 79, 472–477.

## SELECTED REFERENCES FOR DIVERSITY STUDIES

- <sup>1</sup>Caldeira, M.C., Ryel, R.J., Lawton, J.H. & Pereira, J.S. (2001). Mechanisms of positive biodiversity–production relationships: insights provided by delta C-13 analysis in experimental Mediterranean grassland plots. *Ecol. Lett.*, 4, 439–443.
- <sup>2</sup>Crawley, M.J., Brown, S.L., Heard, M.S. & Edwards, G.R. (1999). Invasion-resistance in experimental grassland communities: species richness or species identity? *Ecol. Lett.*, 2, 140–148.
- <sup>3</sup>Foster, B.L. Smith, V.H., Dickson, T.L. & Hildebrand, T. (2002). Invasibility and compositional stability in a grassland community: relationships to diversity and extrinsic factors. *Oikos*, 99, 300–307.
- <sup>4</sup>Fridley, J.D. (2002). Resource availability dominates and alters the relationship between species diversity and ecosystem productivity in experimental plant communities. *Oecologia*, 132, 271–277.

## SELECTED REFERENCES FOR ENDOPHYTES

- <sup>A</sup>Bradshaw, A.D. (1959). Population differentiation in *Agrostis tenuis* Sibth II. The incidence and significance of infection by *Epicblöe typhina*. *New Phytol.*, 58, 310–315.

- <sup>5</sup>Hector, A., Bazeley-White, E., Loreau, M., Otway, S. & Schmid, B. (2002). Overyielding in grassland communities: testing the sampling effect hypothesis with replicated biodiversity experiments. *Ecol. Lett.*, 5, 502–511.
- <sup>6</sup>Hooper, D.U. & Vitousek, P.M. (1997). The effects of plant composition and diversity on ecosystem processes. *Science*, 277, 1302–1305.
- <sup>7</sup>Klironomos, J.N., McCune, J., Hart, M. & Neville, J. (2000). The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. *Ecol. Lett.*, 3, 137–141.
- <sup>8</sup>Knops, J.M.H., Tilman, D., Haddad, N.M. *et al.* (1999). Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecol. Lett.*, 2, 286–293.
- <sup>9</sup>Levine, J.M. (2000). Species diversity and biological invasions: relating local process to community pattern. *Science*, 288, 852–854.
- <sup>10</sup>Lyons, K.G. & Schwartz, M.W. (2001). Rare species loss alters ecosystem function – invasion resistance. *Ecol. Lett.*, 4, 358–365.
- <sup>11</sup>Mitchell, C.E., Tilman, D. & Groth, J.V. (2002). Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. *Ecology*, 83, 1713–1726.
- <sup>12</sup>Mulder, C.P.H., Koricheva, J., Huss-Danell, K., Hogberg, P. & Joshi, J. (1999). Insects affect relationships between plant species richness and ecosystem processes. *Ecol. Lett.*, 2, 237–246.
- <sup>13</sup>Naeem, S., Knops, J.M.H., Tilman, D., Howe, K.M., Kennedy, T. & Gale, S. (2000). Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. *Oikos*, 91, 97–108.
- <sup>14</sup>Naeem, S., Thompson, L.J., Lawler, S.P., Lawton, J.H. & Woodfin, R.M. (1994). Declining biodiversity can alter the performance of ecosystems. *Nature*, 368, 734–737.
- <sup>15</sup>Špačková, I. & Lepš, J. (2001). Procedure for separating the selection effect from other effects in diversity–productivity relationship. *Ecol. Lett.*, 4, 585–594.
- <sup>16</sup>Symstad, A.J. (2000). A test of the effects of functional group richness and composition on grassland invasibility. *Ecology*, 81, 99–109.
- <sup>17</sup>Symstad, A.J., Tilman, D., Willson, J. & Knops, J.M.H. (1998). Species loss and ecosystem functioning: effects of species identity and community composition. *Oikos*, 81, 389–397.
- <sup>18</sup>Tilman, D., Lehman, C.L. & Thomson, K.T. (1997). Plant diversity and ecosystem productivity: theoretical considerations. *Proc. Natl Acad. Sci. USA*, 94, 1857–1861.
- <sup>19</sup>Tilman, D. (1996). Biodiversity: population versus ecosystem stability. *Ecology*, 77, 350–363.
- <sup>20</sup>Wilsey, B.J. & Potvin, C. (2000). Biodiversity and ecosystem functioning: importance of species evenness in an old field. *Ecology*, 81, 887–892.

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